TESIS DE DOCTORADO

DEVELOPMENT OF ENVIRONMENTALLY FRIENDLY COMPOSITE FILMS BASED ON CELLULOSE

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Development of environmentally friendly composite films based on cellulose

Dña. PATRICIA CAZÓN DÍAZ

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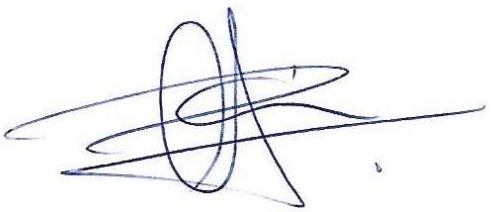

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Development of environmentally friendly composite films based on cellulose

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En Lugo, 20 de febrero de 2020.

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Agradecimientos

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A Rafael y Paco

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FOREWORD

This document was written following the regulations of the University of Santiago de Compostela for the presentation of Doctoral Thesis by a brief summary of articles, being divided into the following sections:

– "**Resumen**", an extensive abstract in Spanish is included following the requirements for doctoral theses written in English.

– **Introduction**, which contains the state-of-the-art of the topic and the justification and methodology of the research.

– **Objectives**, where the general and specific objectives of the work are explained.

– **Results and general discussion** section, which summarizes and relates the results obtained and presented in the articles included in the annexes.

– General **conclusions** of all the research.

– **Bibliography**, which includes the bibliographic references cited in the Introduction section.

– **Publications quality criteria**, which indicates the data of the publications and manuscripts products of the doctoral thesis

research and information of the journals (editor, country, ISSN, journal metrics).

– **Annexes**, which includes an integral copy of the publications and manuscripts resulted from the work carried out during the doctoral program and the academic tutoring. The annexes include 5 published articles and 2 manuscripts submitted at the time that the thesis is deposited. The annexed publications in this section are:

- **Annex I:** Novel composite films from regenerated cellulose - glycerol - polyvinyl alcohol: Mechanical and barrier properties.
- **Annex II:** Cellulose - glycerol - polyvinyl alcohol composite films for food packaging: Evaluation of water adsorption, mechanical properties, light-barrier properties and transparency.
- **Annex III:** Characterization of mechanical and barrier properties of bacterial cellulose, glycerol and polyvinyl alcohol (PVOH) composite films with eco-friendly UV-protective properties.
- **Annex IV:** Composite Films with UV-Barrier Properties of Bacterial Cellulose with Glycerol and Poly(vinyl alcohol): Puncture Properties, Solubility, and Swelling Degree.
- **Annex V:** Regenerated cellulose films combined with glycerol and polyvinyl alcohol: Effect of moisture content on the physical properties.

- **Annex VI:** Bacterial cellulose films: evaluation of the water interaction.
- **Annex VII:** UV-Protecting films based on bacterial cellulose, glycerol and polyvinyl alcohol: effect of water activity on barrier, mechanical and optical properties.

This document does not include a specific section of "Materials and methods", because this section is included in each publication presented.

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RESUMEN

Los envases desempeñan un papel fundamental en la conservación de los alimentos, actuando como barrera física para proteger el producto de una posible contaminación ambiental, daños físicos u otros factores externos, como gases, olores, luz y humedad. Los envases son una herramienta fundamental para garantizar la calidad y la seguridad de los alimentos, a la vez que permiten aumentar su vida útil y disminuir el desperdicio alimentario a lo largo de la cadena de distribución.

En la sociedad actual los envases forman parte de la vida cotidiana de todas las personas alrededor del mundo, como reflejan los datos de producción y consumo publicados en diversos estudios. En el año 2015, el mercado mundial de envases plásticos supuso una producción de aproximadamente 4.300 billones de unidades, de los cuales el 73% procedían de la industria alimentaria. En el año 2018, el consumo de envases procedentes de la industria alimentaria dentro de la Unión Europea supuso aproximadamente 1.130 billones de unidades. El excesivo consumo de estos materiales plásticos no biodegradables y los problemas asociados a su deficiente gestión ha llevado a una generación incontrolada de residuos plásticos no biodegradables en el medio ambiente. En la Unión Europea, la generación de residuos plásticos por

habitante fue de 154,9 kg en el año 2013 y se prevé que seguirá en continuo crecimiento con una tasa anual de 4,2% hasta el año 2024.

El excesivo consumo de envases plástico no biodegradables y su compleja gestión de recuperación o reciclaje han derivado en una acumulación incontrolada en el medio ambiente desencadenando serios problemas medioambientales. La sociedad, concienciada con el impacto medioambiental de los envases plásticos, demanda el desarrollo de nuevas alternativas y materiales biodegradables que puedan sustituir gradual, o al menos, parcialmente a los principales materiales plásticos utilizados en el sector alimentario.

En las últimas décadas, los investigadores han destinado esfuerzos y recursos para desarrollar nuevos materiales con propiedades biodegradables, tanto de origen natural y de fuentes renovables, como materiales sintéticos. Entre los principales materiales estudiados procedentes de fuentes renovables se encuentran los polisacáridos. Muchos polisacáridos como la celulosa, quitosano o almidón, poseen la capacidad de formar películas o recubrimientos inocuos con potenciales aplicaciones sobre alimentos. Estas películas o recubrimientos a base de polisacáridos pueden actuar como un refuerzo de la pared externa del alimento, permitiendo mantener un control sobre el intercambio de vapor de agua, otros gases u olores entre el alimento y el ambiente externo. Además, estas películas pueden proporcionar esterilidad superficial y en algunos casos, protección barrera contra las radiaciones

de luz UV, retardando la aparición de oxidaciones lipídicas, pérdida de vitaminas o pérdida de propiedades organolépticas del alimento.

Entre los polisacáridos que presentan potenciales propiedades para desarrollar nuevos materiales biodegradables se encuentra la celulosa. Ésta constituye el polisacárido más abundante de la Tierra, siendo las plantas la principal fuente. Además, la celulosa es sintetizada por varios microorganismos, conociéndose como celulosa bacteriana (BC). La celulosa vegetal y bacteriana tiene la misma estructura química. Sin embargo, la celulosa de origen vegetal puede contener restos de otros compuestos vegetales, como son la lignina y hemicelulosa, a diferencia de la BC que es pura. A pesar de ser el polímero biodegradable más abundante de la Tierra, la aplicación de materiales biodegradables a base de celulosa no está muy extendido en la industria alimentaria. El principal motivo se debe a una serie de limitaciones que presentan estos materiales, como son las propiedades mecánicas y ópticas entre otras.

Una estrategia ampliamente extendida para mejorar las propiedades de los materiales biodegradables de origen natural y superar determinadas limitaciones es la combinación de diferentes materiales biodegradables. El objetivo es lograr materiales compuestos con mejoradas propiedades funcionales con respecto a los polímeros puros empleados. De acuerdo con esto, la combinación de celulosa con otros polímeros biodegradables o agentes plastificantes ofrece un método simple para desarrollar nuevos materiales compuestos con propiedades funcionales mejoradas.

El alcohol polivinílico (PVOH) es un polímero sintético, soluble en agua y biodegradable, con buenas propiedades elásticas y ópticas ampliamente estudiado como agente reforzante y/o plastificante en otros polisacáridos como quitosano y almidón. La combinación de celulosa con PVOH permite mejorar las propiedades mecánicas y ópticas de las películas de celulosa pura. Además, al tratarse de un polímero soluble en agua, puede incorporarse a la matriz polimérica mediante inmersión de películas de celulosa en baños con una determinada concentración de PVOH.

Por otro lado, el glicerol es un plastificante comúnmente utilizado en el desarrollo de materiales naturales biodegradables. La combinación de celulosa con glicerol y PVOH puede resultar en películas combinadas con propiedades de resistencia, elasticidad y ópticas mejorada con respeto a las películas de celulosa pura.

El estudio de las propiedades funcionales del material desarrollado es fundamental para conocer el comportamiento del material e idoneidad en función de la aplicación final o determinar potenciales aplicaciones. Además, esta evaluación permite analizar el efecto y la interacción entre los componentes del material que facilitará hacer futuras reformulaciones para mejorar o adaptar las propiedades del polímero en función de las necesidades.

Entre los principales estudios para la caracterización de un nuevo material polimérico están el análisis de las propiedades mecánicas, permeabilidad al vapor de agua (WVP), capacidad de retención de agua

(%W), solubilidad (%S), protección barrera contra la radiación UV y transparencia de la película.

Por otro lado, es importante tener en cuenta que tanto la celulosa como el glicerol y PVOH poseen naturaleza hidrófila, siendo sensibles a la humedad ambiental. El contenido de humedad de estos materiales depende de las condiciones de humedad relativa (RH) del ambiente en el que se encuentran. Las moléculas de agua adsorbidas en el material actúan como agente plastificante, por lo que las propiedades del polímero van a depender del contenido de humedad y a su vez de las condiciones de RH del ambiente que los rodea. Los estudios de contenido de humedad de las películas desarrolladas y sus propiedades en función de las condiciones ambientales son fundamentales para tener una completa caracterización del material y conocer su comportamiento e idoneidad en función de las condiciones en las que se aplicará o a las que se destinará el material.

El análisis de las isotermas de adsorción de humedad permite relacionar el contenido de humedad de la película en función de las condiciones de RH o actividad de agua de la muestra (a_w), ya que en condiciones de equilibrio de humedad, la a_w del material se equipara a la RH del entorno circundante.

Por lo tanto, el objetivo de esta tesis de doctorado fue analizar y caracterizar las propiedades de películas a base de celulosa, tanto de origen vegetal como bacteriano, combinadas con glicerol y PVOH. Se analizó la interacción entre los componentes de la película y el efecto

de cada uno en las propiedades finales del compuesto. Se obtuvieron modelos matemáticos predictivos que permiten describir el efecto de los componentes de la película en las propiedades analizadas. Mediante microscopía electrónica de barrido (SEM), espectrofotómetro UV-VIS, espectroscopía infrarroja por transformación de Fourier (FT-IR) y calorimetría diferencial de barrido (DSC), se analizaron las propiedades morfológicas, ópticas, estructurales y térmicas del compuesto. El conjunto de experimentos siguió el diseño Box-Behnken y el análisis estadístico se realizó utilizando el software Design Expert® 10.0.6.

A partir de los resultados obtenidos, se seleccionó la formulación de celulosa regenerada-glicerol-PVOH y celulosa bacteriana-glicerol-PVOH con las propiedades más idóneas y se evaluó las isotermas de adsorción de agua y el efecto del contenido de humedad en las propiedades mecánicas, de permeabilidad y ópticas de las películas desarrolladas. En este caso, el análisis de varianza (ANOVA) se utilizó para evaluar el efecto de la condición a_w en las propiedades estudiadas. La comparación de medias se realizó mediante la prueba de Tukey ($p < 0.05$) usando Statistica 7.0. Se utilizó Microsoft Excel® para calcular la isoterma de adsorción y ajustar el modelo GAB por regresión no lineal.

En el caso de películas a partir de celulosa de origen vegetal, las películas se obtuvieron a partir de celulosa microcristalina usando el método de disolución de celulosa NaOH/urea seguido de un proceso de regeneración por inmersión en un baño de ácido acético al 1%(v/v). El

glicerol se adicionó a la suspensión transparente de celulosa en NaOH/urea hasta alcanzar una concentración final entre 0-5% (p/p). Las películas de celulosa regenerada obtenidas se combinaron con PVOH mediante inmersión en baños con una concentración de PVOH entre 2-10% (p/p).

Las propiedades mecánicas se analizaron con un texturómetro. El análisis de tensión se llevó a cabo siguiendo la norma ASTM D-882 para calcular las propiedades de resistencia a la tensión (TS), porcentaje de elongación (%E) y tenacidad (T). Los valores de TS calculados se encontraron el rango 25,90-368,82 MPa. La resistencia a la tensión disminuyó al aumentar la concentración de celulosa, probablemente debido al incremento de las regiones cristalinas. El PVOH reforzó la matriz de celulosa, mejorando así la resistencia a la rotura. El efecto del glicerol en las propiedades de resistencia dependió de la concentración de celulosa y PVOH, y sus interacciones.

Las muestras mostraron una elasticidad comprendida entre 0,89-18,74%. Las propiedades elásticas dependieron de la concentración de PVOH y las interacciones entre glicerol y PVOH. El PVOH actuó como agente plastificante en la matriz de celulosa. Sin embargo, el glicerol manifestó un efecto "antiplastificante" en la película, disminuyendo la elasticidad de la muestra al aumentar la concentración de glicerol.

Los valores T obtenidos se encontraron entre 0,25-44,30 MJ/m^3. El PVOH manifestó un efecto reforzante de la matriz, aumentando los valores de T al aumentar su concentración. A diferencia del glicerol,

que dificultó la movilidad de las cadenas poliméricas produciendo la disminución de los valores de T al aumentar la concentración de glicerol.

Las propiedades de punción se midieron usando un texturómetro con un soporte de película HDP/FSR. Con las pruebas de punción se obtuvieron los valores de resistencia a la punción (BS) y elasticidad de punción (DB). Los resultados de la prueba de punción mostraron valores de BS en el rango de 1395 a 12014 g y DB en el rango de 1.60 a 6.04 mm. En ambos casos, el efecto lineal de la concentración de PVOH es el único término significativo. Este comportamiento se debe probablemente al efecto plastificante y de refuerzo de PVOH en la matriz de celulosa, facilitando la movilidad de las cadenas durante la deformación.

Los valores de WVP de las películas se midieron siguiendo el método gravimétricamente ASTM E96. Los valores de permeabilidad de las muestras se encontraron entre $2.32\text{-}10^{-11}\text{-}3.01\text{-}10^{-11}$g/s·m·Pa. El aumento de la concentración de la celulosa contribuyó a la disminución de los valores de permeabilidad, probablemente debido al incremento de las regiones cristalinas en la matriz. El carácter hidrófilo del glicerol resultó en un aumento de la permeabilidad de la película. Los valores de permeabilidad obtenidos para las películas desarrolladas fueron de 2 órdenes de magnitud superiores a los valores de WVP de polímeros sintéticos. Sin embargo, las películas compuestas de celulosa-PVOH

mostraron valores de permeabilidad inferiores a los publicados para películas puras de PVOH.

El %W se calculó a partir de la diferencia de peso entre muestras húmedas, previamente sumergidas en agua destilada durante 24 h, y el peso de muestras secas. Los valores de %W calculados oscilaron entre136.35 a 222.09%. La presencia de PVOH aumentó los valores de %W, sin variaciones significativas dependiendo del contenido de celulosa y glicerol.

Las propiedades ópticas de las películas de celulosa regenerada con glicerol y PVOH se midieron utilizando un espectrofotómetro V-670 en modo de transmitancia en las regiones UV-VIS-NIR. La transparencia de las películas se calculó a partir del valor de porcentaje de transmitancia medido a una longitud de onda de 600 nm. Los resultados indicaron que el glicerol es capaz de disminuir los valores de transmitancia de la película en la región UV, mejorando las propiedades de barrera a la radiación UV. Sin embargo, la presencia de PVOH aumentó estos parámetros, teniendo un efecto positivo en la región visible, al mejorar los parámetros de transparencia de la muestra.

La interacción entre los componentes del polímero compuesto se analizó mediante imágenes SEM, FT-IR y DSC. Según las imágenes SEM, la presencia de PVOH redujo la rugosidad y porosidad de la película de celulosa, obteniendo una película más compacta y densa. FT-IR y DSC confirmaron la interacción entre los componentes de las películas y la retención de glicerol en la matriz después de los procesos

de lavado. El análisis térmico de las muestras indicó que el glicerol y el PVOH aumentaron la estabilidad térmica de las películas, en respuesta a la interacción entre los componentes.

Los resultados obtenidos en esta tesis de doctorado indicaron que las películas con una composición de celulosa 4% (p/p), glicerol 5% (p/p) y PVOH 5% (p/p) tienen buenos valores de elasticidad, resistencia a la rotura, barrera UV y propiedades de adsorción. Por lo tanto, se seleccionó esta formulación para estudiar las isotermas de adsorción de humedad y evaluar el efecto plastificante de las moléculas de agua en las propiedades de películas de celulosa regenerada combinadas con glicerol y PVOH.

Para lograr diferentes valores de a_w en las películas y poder evaluar sus propiedades en función del contenido de humedad, se recrearon microclimas con diferentes valores de RH en envases herméticamente cerrados. Las películas se acondicionaron en estos microclimas a temperatura contante y el tiempo suficiente para alcanzar el contenido de humedad de equilibrio en cada muestra. El modelo matemático GAB se utilizó para obtener la isoterma de adsorción de humedad a partir de los datos experimentales.

Los datos de adsorción experimental se ajustaron bien utilizando la ecuación GAB (r^2> 0,99) y la isoterma de adsorción de humedad de las películas de celulosa regenerada-glicerol-PVOH obtenida fue del tipo II. Los parámetros de GAB calculados fueron X_m=4.16, C=19.91 y K=0.89. Las propiedades de permeabilidad de las muestras dependieron

del contenido de humedad, aumentando al aumentar el contenido de humedad, observando valores de permeabilidad entre $5.15 \cdot 10^{-10}$ y $5.44 \cdot 10^{-9}$ g/m·s·Pa, dependiendo de la a_w.

El análisis de las propiedades mecánicas de las películas con diferentes contenidos de humedad evidenció el efecto plastificante del agua en la película, disminuyendo la resistencia a la rotura y aumentando la elasticidad del compuesto polimérico a medida que aumentaba a_w. Los datos obtenidos estuvieron en los intervalos siguientes: 92,65-17,57 MPa para TS, 5,88-19,85% para %E y (3639,09-227,89 MPa para T. Sin embargo, a elevados contenidos de humedad se observó una "sobreplastificación" de la película, que debilitó la matriz polimérica disminuyendo su elasticidad hasta 10,76%.

En las pruebas de punción se obtuvieron resultados de BS y DB en los intervalos siguientes: 525,47-3420,18 g y 0,59-3,97 mm, respectivamente. En este caso, el agua adsorbida actúo como lubricante facilitando la reorganización de las cadenas del polímero durante la deformación bajo fuerzas de punción, aumentando la resistencia y elasticidad al aumentar el contenido de agua de la película.

En cuanto a las propiedades ópticas, el efecto plastificante de las moléculas de agua afectó a las propiedades ópticas de las películas de celulosa regenerada celulosa-glicerol-PVOH. A elevados valores de a_w, el agua condensada en la matriz disminuyó los valores de transmitancia en la región UV.

La caracterización de películas a base de celulosa de origen bacteriano combinadas con glicerol y PVOH se realizó siguiendo la misma metodología mencionada. Las películas de BC puras se obtuvieron directamente por fermentación en medio líquido de la bacteria *Komagataeibacter xylinus*, usando glucosa como fuente de carbono. Las películas de BC se combinaron con glicerol y PVOH por inmersión en baños con disoluciones de glicerol y PVOH con concentraciones entre 0-5% (p/p). En este caso, no fue necesario un proceso de disolución y regeneración de la celulosa, porque se obtuvo una película directamente del medio de cultivo.

En este caso, se analizó el contenido de humedad de muestras de cada formulación en condiciones estándar de análisis de las propiedades mecánicas. Los datos mostraron una oscilación en el contenido de humedad de las muestras entre 1,82 y 91,10%, en función su concentración. El glicerol mostró un mayor efecto en el incremento del contenido de humedad de las muestras que el PVOH, debido a la naturaleza higroscópica del plastificante.

En cuanto a las propiedades mecánicas, los valores calculados variaron entre 1,23 y 35,05 MPa para de TS, entre 2,28 y 49,89% para %E, entre 10,84 y 1489,15 MPa para los valores del módulo de Young (YM) y entre 0,22 y 2,60 MJ/m^3 para T. Los resultados indicaron que el PVOH actúo como agente reforzante al penetrar en la estructura de BC, mientras que el glicerol actuó como plastificante. Además,

muestras con glicerol contenían mayor cantidad de agua, que desempeña un papel plastificante en la matriz de celulosa.

La prueba de punción mostró unos valores de resistencia a la punción entre 43,74 y 2105,52 g, y de DB entre 0,39 y 4,94 mm. El PVOH y glicerol mostraron un efecto similar al observando en las pruebas de punción.

Las diferentes formulaciones de BC-glicerol-PVOH mostraron valores de permeabilidad entre $1.87\text{-}10^{-11}$ y $2.04\text{-}10^{-10}$ g/m·s·Pa. A pesar de la naturaleza hidrófila del PVOH, el aumento de concentración de PVOH en la película resultó en una disminución de la permeabilidad. Como se observó en las imágenes SEM, la incorporación de PVOH en la matriz de celulosa supuso una importante reducción de la porosidad de la película y un aumento de la densidad, dando una película más compacta, y dificultando así la difusión de vapor de agua a través de la matriz.

Las películas de BC-glicerol-PVOH mostraron unos valores de capacidad de retención de agua entre 78,26 y 364,78% en función de la composición. Las películas de celulosa pura mostraron mayor capacidad de retención de agua debido a la estructura tridimensional de la película que facilitó la penetración y retención de las moléculas de agua. La presencia de PVOH y glicerol disminuyeron la capacidad de retención de agua de la película, principalmente debido a la disminución de la porosidad. En cuanto a la solubilidad de las muestras, después de estar sumergidas durante 24 h en agua destilada con agitación, los

valores calculados por diferencia de pesada oscilaron entre 9,37 y 31,65%.

Los espectros de transmitancia en la región UV señalaron que las películas de BC poseen óptimas propiedades de barrera contra la radiación UV. Estas propiedades mejoraron con la presencia de glicerol. En cuando al PVOH, ayudó a mejorar las propiedades en la región visible, obteniendo películas más transparentes y menos opacas.

Las interacciones entre BC, glicerol y PVOH se analizaron mediante imágenes SEM, FT-IR y análisis TGA-DSC. Las imágenes SEM mostraron la estructura porosa de las películas de BC, formada por fibrillas que forman láminas superpuestas. El PVOH y glicerol difunden a través de los poros, resultando en una estructura más compacta, densa y gruesa.

Las técnicas FT-IR y TGA-DSC evidenciaron la interacción entre los componentes del polímero compuesto y el aumento de la estabilidad térmica del compuesto BC-glicerol-PVOH.

A partir de los resultados obtenidos en el estudio de películas a base de BC combinada con glicerol y PVOH, se seleccionó la formulación que mostró mejores propiedades mecánicas, siendo las películas con una concentración de glicerol y PVOH del 5% (p/p).

La curva de la isoterma de adsorción de BC-glicerol-PVOH fue de tipo III, característica de películas que contienen componentes hidrofílicos como el glicerol. Los datos experimentales de adsorción de humedad se ajustaron bien para obtener la isoterma utilizando la

ecuación de GAB (r^2 > 0,99). Los valores de los parámetros de GAB calculados fueron X_m=13,80, C=1,17 y K=0,91.

El estudio de la permeabilidad de las películas en un amplio rango de RH demostró que la permeabilidad depende de la a_w y la naturaleza higroscópica del glicerol, con valores que oscilaron entre $6.50 \cdot 10^{-9}$ y $2.71 \cdot 10^{-8}$ g/m·s·Pa.

La resistencia a la rotura de las muestras demostró una disminución continua al aumentar el contenido de humedad, como resultado del efecto plastificante del agua, con valores de TS entre 1,16 y 6,57 MPa y YM entre 10,31 y 70,54 MPa. En cuanto a la elasticidad, las muestras mostraron valores elásticos entre 8,51-34,08%. El efecto plastificante del agua se observó a bajos valores de a_w (a_w<0.32), aumentando la elasticidad de las muestras. Sin embargo, a elevados valores de a_w, se observó un efecto "sobreplastificante" que debilitó la estructura de la película disminuyendo su elasticidad. La resistencia a la punción (299,92-1943,26 g) y elasticidad (2,56-4,03) mostraron una tendencia similar a los valores de la prueba de tensión.

Las propiedades de barrera a la radiación UV de las películas desarrolladas analizadas a varias a_w indicaron la disminución de la transmitancia de las películas al aumentar el contenido de humedad de las muestras, mejorando las propiedades de protección contra la radiación UV. A elevados valores de a_w, las muestras se volvieron ligeramente opacas, probablemente debido a las propiedades higroscópicas del glicerol.

Los resultados obtenidos demostraron que, siguiendo un sencillo método de inmersión, es posible modificar las propiedades de las películas de BC gracias a su estructura tridimensional y porosa. Este hecho abre la puerta a futuras reformulaciones de películas a base de BC con potenciales aplicaciones en el área de tecnología de los alimentos, medicina, cosmética, etc. Por este motivo, se analizaron las isotermas de adsorción de humedad y el efecto del agua en las propiedades funcionales de películas de BC pura. Los datos experimentales de adsorción de humedad se ajustaron bien a la ecuación de GAB (r^2>0.98). Los parámetros obtenidos fueron X_m=3.82, C=12.00 y K=0.95. La curva de la isoterma de adsorción de BC se clasificó como tipo II, con tres regiones principales.

Los valores de permeabilidad de BC dependieron de la a_w de la película, oscilando entre $1.35 \cdot 10^{-12}$ y $3.13 \cdot 10^{-11}$ g/m·s·Pa en función del contenido de humedad de la muestra. La permeabilidad de la película aumentó significativamente al aumentar la a_w, hasta 0,48 a $_w$. A valores superiores de a_w se observó una relativa estabilización de la permeabilidad.

Al aumentar las condiciones de RH, las moléculas de agua adsorbidas en la película de celulosa manifestaron un efecto plastificante observado en las propiedades mecánicas, tanto de tensión como de punción. Por lo tanto, el comportamiento mecánico de las películas de BC dependen del contenido de humedad de la muestra. Como resultado, el aumento del contenido de humedad de las películas

redujo la resistencia a la tensión del polímero, mejorando la elasticidad y la resistencia a la punción. Los valores obtenidos de las propiedades mecánicas en las pruebas de tensión estuvieron en los intervalos 15,50-22,28 MPa para TS, 1,36-3,71% para %E y 609,12-1426,77 MPa para YM. Los resultados de las pruebas de punción indicaron valores de BS entre 145,03 y 338,10 g y de DB entre 0,36 y 1,86 mm.

El espectro en la región UV de las películas de BC mostraron bajos valores de transmitancia, indicado la capacidad de protección de la celulosa contra las radiaciones UV. Elevados contenidos de humedad disminuyeron los valores de transmitancia de la película.

Los resultados obtenidos indicaron que es factible desarrollar películas a base de celulosa con propiedades mejoradas mediante un sencillo método de combinación con PVOH y glicerol. Las películas desarrolladas presentan potenciales aplicaciones como películas de recubrimiento de alimentos, con idóneas propiedades de barrera contra la radiación UV.

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CHAPTER I – INTRODUCTION

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1. FOOD PACKAGING

In today's society, packaging is essential. It is part of the daily life of every person worldwide. In 2015, the global packaging market was estimated at 4300 billion units of packages, which 73% were for food packaging. In 2018, 1130 billion food packages were used in the European Union. Since 2010, waste production has increased at an annual rate of 4.2% and it is estimated to continue with the same growth until 2024 (Ketelsen, Janssen, & Hamm, 2020). The high consumption of packages is accompanied by a large generation of waste. It was estimated that 156.9 kg of packaging waste per inhabitant was generated in the European Union in 2013 (Licciardello, 2017).

Packaging plays a fundamental role in food preservation, acting as a physical barrier to protect the product against environmental contamination and other external factors, such as, gases, odors, light, water vapor or physical damages (Bopp, 2019; Han, Ruiz-Garcia, Qian, & Yang, 2018). It is a fundamental tool to guarantee the food quality and safety, while extending the shelf-life of products and minimizing food waste along the distribution chain (handling, transporting and storage) (Han et al., 2018; Robertson, 2012). Furthermore, the

packaging has a fundamental role as a means to give information about the packed product and it shows a psychological impact on a potential customer (Rodríguez-Rojas, Arango Ospina, Rodríguez-Vélez, & Arana-Florez, 2019). Over the years, food packaging evolved based on advances in knowledge and understanding of food spoilage to improve packaging functionality (Bopp, 2019).

Several materials have been used as packaging: natural materials, ceramics, paper products, glass, metals, plastics and polymer films (Bopp, 2019; Han et al., 2018). However, since the 1970s the production and applications of synthetic films have grown quickly, being the most widely used materials for packaging purposes. Global plastic production in 2017 reached over $335 \cdot 10^6$ tons, a significant increase with respect to the $5 \cdot 10^6$ tons produced in the 1950s (Napper & Thompson, 2019; Robertson, 2012; Tessnow-von Wysocki & Le Billon, 2019).

The significant increase in the use of synthetic plastics is due to the excellent properties of these materials at a relative low cost, such as excellent oxygen/moisture barrier properties, visual appearance, heat sealability and excellent mechanical properties suitable to keep the integrity of the packaging during its useful life (Cazon, Velazquez, Ramírez, & Vázquez, 2017; Trinetta, 2016). The most commonly used polymers are (Bopp, 2019; Robertson, 2012):

– High-density polyethylene (HDPE) is used to contain liquid food products, such as milk or juice.

– Low-density polyethylene (LDPE) is less rigid than HDPE and finds applications in films and squeeze bottles.

– Very low-density polyethylene (VLDPE) is mostly used for the packaging of fresh products, milk, meat and cheese, as well as for the manufacture of multilayer coextruded films.

– Polyethylene terephthalate (PET) is used in carbonated beverage bottles.

– Polyvinyl chloride (PVC) is used in plastic tubes and film wrappers. It is resistant to attack by oils, so it has also applications in cooking oil containers. In recent years, PVC has been increasingly replaced by PET.

– Polystyrene (PS) is used for disposable packages such as meat and product trays, egg cartons, disposable tableware and containers for take-out or take-out meals.

– Polypropylene (PP) is used in single-serving tubs, such as yogurt containers and microwavable packaging.

These materials represent approximately 90% of total plastic production (Napper & Thompson, 2019). Despite their durability, the main use as packaging is in relatively short-lived and single-use, being around 40% of all plastic production (Napper & Thompson, 2019). The short shelf-life of plastic packaging, its complex waste management and its non-biodegradability have led to a serious environmental problem

due to its uncontrollable accumulation in the environment. It is estimated that $(4.8\text{-}12.7)\cdot 10^6$ ton of plastic reached the oceans from land in 2010, being 75% of all marine litter (Andrady, 2011; Jambeck et al., 2015; Napper & Thompson, 2019).

Actually, recycling is considered the main mechanism to reduce environmental and waste management problems associated with the use of plastics. However, several limitations hinder the recycling of these materials. Some plastic products are considered too small for a practical classification. For example, less than 3% of the 500 billion plastic bags that are distributed in the market every year are recycled (Trinetta, 2016). Besides, some packaging products are prone to contamination with organic (particularly food) or chemicals products. For example, PS trays that are commonly used for meat packaging cannot be recycled due to the meat exudates absorbed into the polymer matrix. Other important limitation are materials that are a combination of different types of plastic, whether blended polymer or multi-layered materials (with different types of plastic in each layer). The used of multi-layered materials is widespread to improve the gas and moisture barrier properties than single-layer materials. As result, the cost and technology limitations of separating and recycling the different plastic polymers can be prohibitive and alternative strategies are required to address the waste management challenges associated with plastic disposal (Dilkes-Hoffman et al., 2018; Licciardello, 2017).

On the other hand, there is a growing environmental concern about microplastics. Uncontrolled plastic waste exposed to UV-light radiation from sunlight can initiate a degradation process. Once degradation is initiated, it can proceed through temperature-dependent thermo-oxidative reactions without further exposure to UV-light radiation, as long as oxygen is available. As result, this process causes the reduction of plastic size, turning it into small fragments (Napper & Thompson, 2019). The small plastic fragments can be ingested by lower trophic fauna, incorporating into the food-chain with uncertain consequences for the health of the organism and humans (Andrady, 2011; Cole, Lindeque, Halsband, & Galloway, 2011; Jambeck et al., 2015). Recent studies have already shown that the presence of microplastics in food products for human consumption is a fact. It was observed the presence of microplastic in commercial table salts (Iñiguez, Conesa, & Fullana, 2017), seafood (Devriese et al., 2015; Van Cauwenberghe & Janssen, 2014) and freshwater systems (Eerkes-Medrano, Thompson, & Aldridge, 2015).

As result of environmental problems arising from the uncontrolled use and management of non-biodegradable synthetic plastics, and the limitations of the recycling processes of these materials, society demands biodegradable materials as an alternative to several plastic products, including food packaging. In response to this need, in recent decades researchers have focused efforts on developing innovations in the food packaging field. Since the first article published in 1935 on

food packaging materials (Morgan, 1935), there has been an important evolution in the advances and trends in food packaging, with the emergence of intelligent, active, biodegradable, edible or nanotechnology packaging (Embuscado & Kerry, 2009; Ozdemir & Floros, 2004; Rodríguez-Rojas et al., 2019). However, providing sustainable packaging alternatives without compromising the key packaging characteristics (such as barrier properties, mechanical properties and prolonged shelf-life) will require continuous innovation and development in the coming decades(Han et al., 2018).

2. BIODEGRADABLE POLYMERS-BASED FILM FOR FOOD PACKAGING

Biodegradable polymers refer to materials that can be degraded into fragments of lower molecular weight by the action of natural microorganisms such as bacteria, fungi and algae that consume them as a food source. Their original forms are completely degraded to water, CO_2 and biomass. Biodegradation is a relatively rapid process under appropriate conditions of humidity, temperature and available O_2 (Robertson, 2012).

In the last decades, food scientists have been focused on developing new materials with biodegradable properties, mainly based on materials from renewable sources or synthetic polymers. It is important to note that materials from renewable sources do not imply that they are biodegradable. Green-PE or bio-PET obtained from renewable

resources are chemically identical to the conventional polymers. They are non-biodegradable. The biodegradability of a material does not depend on its origin, only on its chemical composition (Licciardello, 2017).

Accordingly, biodegradable polymers can be classified into four main groups as function of their origin and method of production, as shown Figure 1 (Robertson, 2012):

– Polymers directly extracted from biomass.

– Polymers produced by chemical synthesis from biomass monomers.

– Polymers produced directly by natural or genetically modified organisms.

– Polymers obtained from petrochemical-based monomers.

In food packaging field, developing new materials from biomass is gaining attention due to its low cost, no-toxic, edible properties, biocompatible and the opportunity to obtain these polymers from waste or by-products of agriculture and food industry. Proteins and polysaccharides are the most widely investigated biopolymers in the field of edible coating and edible films (Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011).

The use of polysaccharides and/or proteins as raw material for coating or wrapping food, allows the use of safe and edible components to extend the shelf life of the product. These coatings or wraps can be

consumed along with food or discarded. In addition, these components allow to respond to the request of consumers to use natural components for food preservation. Because, edible films provide replacement and/or fortification of the natural layers of the food to avoid moisture losses, controlled exchange of important gases (O_2, CO_2, ethylene) involved in maturation or preservation process, protection against UV-light radiations that affect the organoleptic properties of food and other external odors. In addition, these edible films can provide surface sterility and prevent the loss of other important components (Embuscado & Kerry, 2009).

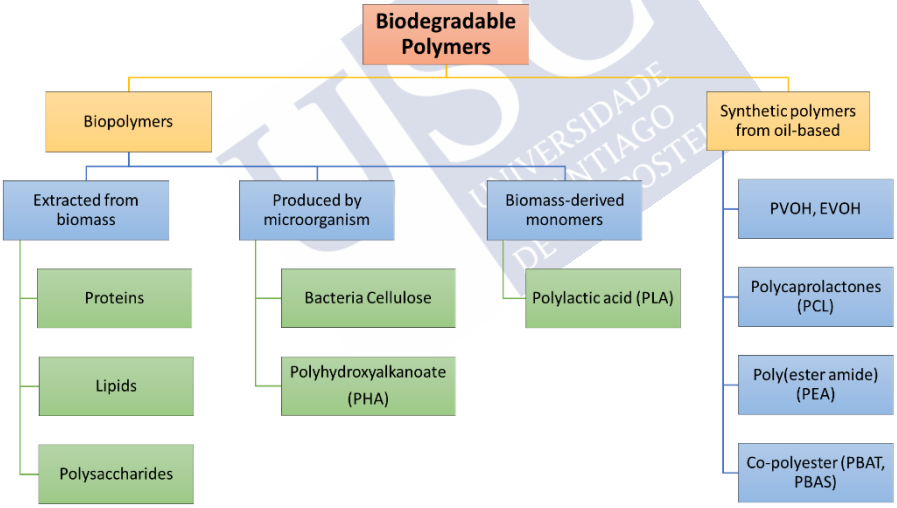

Figure 1. Scheme of types of biodegradable polymers depending on their origin. Adapted from (Robertson, 2012).

2.1. POLYSACCHARIDES-BASED FILMS

Polysaccharides materials, such as chitosan, starch, gums, cellulose and its derivatives have been studied and evaluated as raw material to prepare edible coatings and films to apply as packaging material for food preservation (Aider, 2010; Elsabee & Abdou, 2013; Espitia, Du, Avena-Bustillos, Soares, & McHugh, 2014; Han, 2014; Jiménez, Fabra, Talens, & Chiralt, 2012; Klemm, Heublein, Fink, & Bohn, 2005; Psomiadou, Arvanitoyannis, & Yamamoto, 1996; Vargas & Gonzalez-Martinez, 2010; Zhang, Liu, & Han, 2008). The materials selected to develop new biomaterials must meet properties such as low cost, availability and functional or specific properties (antioxidant, antimicrobial, enriched components). The development of composite polysaccharide-based materials provides the opportunity to market products for a longer shelf-life and ready-to-eat.

One of the most important properties that some polysaccharides have to be studied as films or coatings for food packaging is their film-forming ability (Cazón, Velazquez, Ramírez, & Vázquez, 2017; Elsabee & Abdou, 2013; Espitia et al., 2014).

However, pure polysaccharide films present some limitations, mainly in the water vapour permeability and elasticity of the film. For that reason, combining polysaccharide-based films with other polymers, plasticizers or other components is a strategy to improve the functional properties of the resulted polymer blend with respect to the

pure polymer, keeping their biodegradability. In addition, it is a tool to develop active food packaging with antimicrobial or antioxidant properties.

A polymer blend is defined as the combination of two or more dissimilar polymers by physical mixing with/without chemical interaction between them. In comparison to copolymerization, polymer blends can provide desired properties at a low cost by simple physical processes rather than chemical approaches (Muthuraj, Misra, & Mohanty, 2018).

Generally, the process of preparing the coatings or films requires an initial step of dissolution or dispersion of the polymer, using a solvent such as water, alcohol, mixture of water and alcohol or a mixture of other solvents. Plasticizers, antimicrobial agents, colouring or flavouring agents can be added in this step. Adjusting the pH, heating the solutions and/or other additional steps may be necessary to facilitate the solubility of some biopolymers. From this polymeric solution or suspension, the coating or polymeric film can be obtained depending on how the solution is applied. In most cases, the polymer film is obtained following the casting method. The solution is poured on a flat surface, usually a Petri dish, and dried under specific conditions, allowing evaporation of the solvent and forming the film. Finally, the film is peeled of the plate, as shown Figure 2.

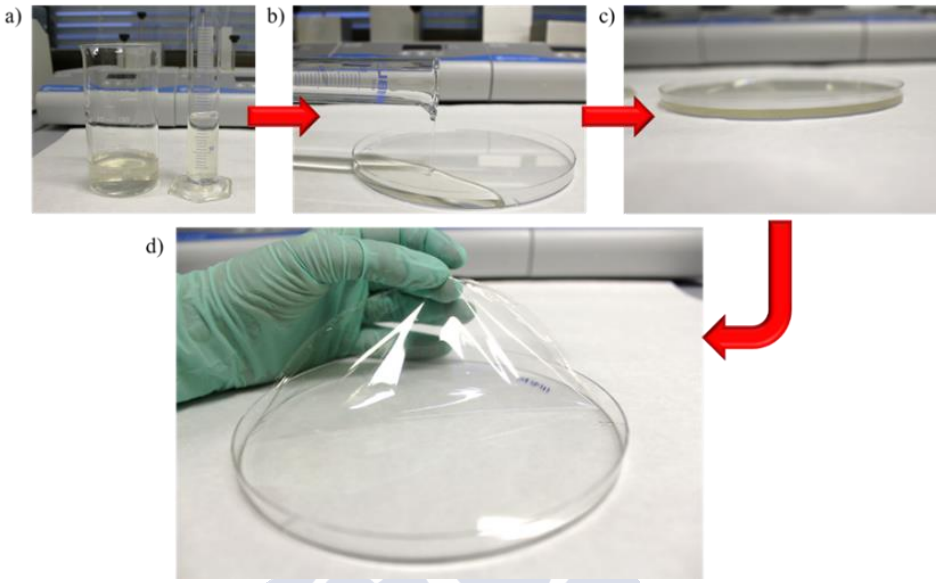

Figure 2. Casting method steps to obtain a chitosan-based film (Cazon & Vázquez, 2019).

In the case of coatings, it is formed on a food by several methods including dipping, spraying, brushing and panning followed by drying (Bourtoom, 2008).

Cellulose is one of the most studied polysaccharides as raw material to develop food packaging films. More than 5000 articles on cellulose films and its cellulose derivates have been published since 2016 until the beginning of 2020 for food packaging applications (ScienceDirect®).

2.1.1. *Cellulose*

Cellulose constitutes the most abundant renewable polymer resource available in nature. It has been widely reported as a raw material for biodegradable films mainly because of its renewability, low cost, non-toxicity, biocompatibility, biodegradability and chemical stability (Wang, Lu, & Zhang, 2016). It is classified as "generally recognized as safe" (GRAS) and approved by the U.S. Food and Drug Administration (FDA) (Jozala et al., 2016). It is also considered as a safe food additive by the European Food Safety Authority (EFSA) (Younes et al., 2018).

It is a linear chain with two anhydroglucose rings ($(C_6H_{10}O_5)_n$) covalently linked through an oxygen in a β-1-4 glycosidic bond (Figure 3) (Klemm et al., 2005). The number of repeated units per chain depends on the source (Wang et al., 2016). The molecules are organized in microfibrils, in which there are extensive hydrogen bonds between cellulose chains, producing a strong crystalline structure.

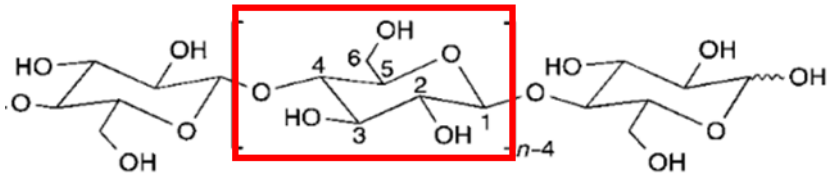

Figure 3. Chemical structure of cellulose, modified from (Klemm et al., 2005)

Although plant is the major contributor of cellulose, being isolated from wood, cotton or hemp (Xu, Chen, Rosswurm, Yao, & Janaswamy, 2016), various microorganisms are able to produce cellulose as an

alternative source. Hence, cellulose is produced by tunicates, several species of algae, and by some species of bacteria including *Acetobacter*, *Agrobacterium*, *Pseudomonas*, *Rhizobium*, *Sarcina*. *Komagataeibacter xylinus*, initially named as *Acetobacter xylinum*, later and most widespread as *Gluconoacetobacter xylinus* (Szymańska-Chargot et al., 2017; Yamada et al., 2012). The extensive renewable sources from which cellulose can be obtained, makes this material an active alternative for natural fillers in plastic materials (Kolybaba et al., 2003).

Vegetable and bacterial cellulose have the same chemical structure. However, cellulose from vegetal sources may contain remains of other natural compounds, like lignin and hemicelluloses. Unlike vegetable cellulose, cellulose synthesized by microorganisms (bacterial cellulose) is pure and free of other compounds or remains (Esa, Tasirin, & Rahman, 2014; Klemm et al., 2005; Nguyen, Gidley, & Dykes, 2008).

In a suitable culture media and conditions, bacterial cellulose is extruded to the surface of the liquid in form of pellicle. The microorganism produces glucose chains from the carbon source contained in the medium, mainly glucose (Cacicedo et al., 2016), although the use of other sugars like fructose as substrate have been reported (Chao, Mitarai, Sugano, & Shoda, 2001). The glucose chains are extruded out through tiny pores present on their cell wall. The microorganism forms microfibrils by combining the glucose chains. The microfibrils are aggregated forming cellulose ribbons, as shown Figure 4. These ribbons form a unique three-dimensional structure

consisting of an ultrafine network of cellulose nanofibers with an expanded surface area and high porosity. The three-dimensional structure of bacterial cellulose with high surface area, porosity, high crystallinity and purity, bacterial cellulose has several advantages over vegetable cellulose, such as higher mechanical properties (Jozala et al., 2016; Rozenberga et al., 2016).

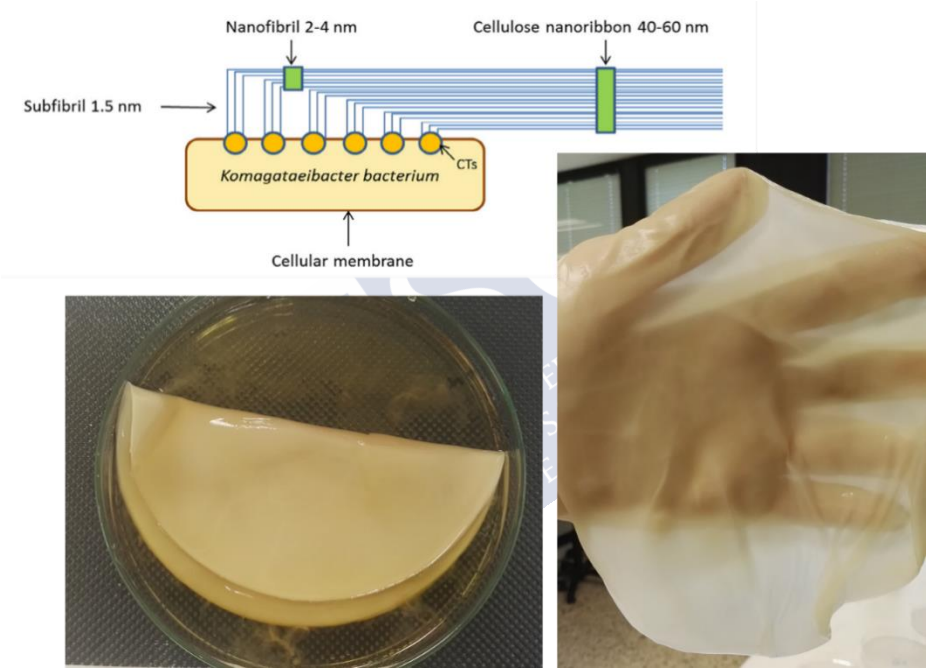

Figure 4. Schematic image of extracellular production of bacterial cellulose, modified from (Cacicedo et al., 2016) and samples of wet bacterial cellulose films.

Cellulose is insoluble in common polar solvents, but it is soluble in a few solvents, which have no similar chemical properties. There are two main hypotheses to justify the insolubility in water of cellulose.

One of the hypotheses supports cellulose insolubility due to the relatively high length of the cellulose chain and its packaging due to the numerous hydrogen bonds along the chain (Wang et al., 2016). Other authors explain the insolubility due to its high crystallinity (Lindman, Karlström, & Stigsson, 2010). Previous researches indicated that hydroxyl groups in C_2, C_3 and C_6 contribute to the formation of various kinds of inter- and intra-molecular hydrogen bonds. The hydrogen bond interactions in the cellulose structure have a strong influence on the physical properties of the polymer (solubility, hydroxyl reactivity, crystallinity) and the mechanical properties (Fan, Dai, & Huang, 2012). The crystallite modulus of native cellulose with and without intramolecular hydrogen bonds were calculated, being 172.9 GPa and 70.8 GPa, respectively (Tashiro & Kobayashi, 1985). Results demonstrated the important role of these hydrogen interactions on the chain deformation mechanism.

As a consequence of its water insoluble property, the direct use of cellulose fibers as film-reinforcing agent to another biopolymer matrix has been extended. It has been studied the reinforcing effect cellulose fiber at several sized on polyvinyl alcohol (Ibrahim, El-Zawawy, & Nassar, 2010; Jalal Uddin, Araki, & Gotoh, 2011; Wahyuningsih, Iriani, & Fahma, 2016), chitosan (Fernandes et al., 2010; A. Khan et al., 2012; Rong, Mubarak, & Tanjung, 2017), starch (Raabe et al., 2015), agar (Atef, Rezaei, & Behrooz, 2015) or alginate-based films (Huq et al., 2012) among other.

However, due the potential properties of cellulose as raw material to develop novel biofilms, several investigations have focused on developing novel methods to dissolve it. Through the dissolution of cellulose, it is possible to use it directly as a polymer matrix. In the literature, it was reported solvents like N-methylmorpholine N-oxide (NMMO), ionic liquids, LiCl/N,N-dimethylacetamide, NaOH aqueous solution, NaOH/urea, NaOH/thiourea aqueous solution, tetra-butylammonium fluoride/dimethyl sulfoxide system, molten inorganic salt hydrates (e.g.: ZnCl2·4H2O, LiI·2H2O, LiBr) and other more complex solutions, like aqueous metal solutions composed of transition metal ions and nitrous ligands, including cuprammonium hydroxide (Cuam: [Cu(NH3)4](OH)2) and cupriethylenediamine hydroxide (Cuen: [Cu(H2N(CH2)2NH2)2](OH)2) (Isik, Sardon, & Mecerreyes, 2014; Lindman et al., 2010; Wang et al., 2016).

Among the solvents developed, NMMO system is the most powerful. However, it has disadvantages of requiring high temperature for dissolution and antioxidants to avoid side reactions of solvents, causing degradation of cellulose, and high costs (S. Zhang, Li, Yu, & Hsieh, 2010). On the other hand, NaOH aqueous system is one of the cheapest cellulose solvents. Cellulose can be dissolved in 7-9% NaOH aqueous solution near 4°C, since intermolecular hydrogen bonds are destroyed. However, it is only used for cellulose with low molecular weight. Hence, NaOH system limits the process, applications, and mechanical properties of cellulose-based products (Zhou & Zhang,

2000). The NaOH system limitations problem were overcome with the addition of urea in the solution. It was observed that cellulose was able to dissolve in NaOH 6% (w/w), but the solutions turned into a gel when the temperature increased. The stability of the solution was improved by adding urea, achieving a good dispersion of the cellulose. (Zhou & Zhang, 2000).

The solvent mechanism of NaOH/urea is based on NaOH can penetrate between crystallites to break inter- and intra-hydrogen bonds between cellulose chains. Urea can prevent the re-association of cellulose molecular chains because acts as a hydrogen bond donor and receptor, improving the stability of the cellulose solution (Zhang et al., 2010; Zhou, Zhang, & Cai, 2004). The NaOH/urea method requires a cooling stage at temperatures below -10ºC to get the solution of cellulose. The effect at low temperatures has been explained by the total enthalpy of cellulose dissolution (Equation 1):

$$\Delta H_{Disolution} = \Delta H_{fusion} + \Delta H_{transition} + \Delta H_{interaction} + \Delta H_{mixing} \quad \text{(Eq. 1)}$$

First, ΔH_{fusion} refers the necessary energy for the transition of the solid polymer to a hypothetical, highly elastic liquid state which corresponds to disintegration of the crystalline regions. $\Delta H_{transition}$ refers to the transition of the amorphous regions from a vitreous to a highly elastic state. Then, $\Delta H_{interaction}$ is the necessary energy to the solvation

of the polymer molecules. Finally, ΔH_{mixing} refers to the mixing of the solvation of the polymer molecules to give an infinitely diluted solution. The only endothermic term in the equation is ΔH_{fusion} associated with the breaking of the hydrogen bonds in the crystalline regions. All other terms are related to interactions between cellulose hydroxyl groups and the solvent system and they are exothermic. The overall process of cellulose dissolution is exothermic and is favored by low temperature (Zhang et al., 2010). During the storage time, cellulose in solution suffers a slight degradation process (Zhou & Zhang, 2000).

Once the cellulose is dissolved in the NaOH/urea solvent, a regeneration process by immersion in a slightly acid bath is necessary to precipitate the polymer and form the film. Cellulose films obtained by this method are named as regenerated cellulose. The acids commonly used in the regeneration bath are acetic acid, H_2SO_4, H_2SO_4/Na_2SO_4, Na_2SO_4 and $(NH_4)_2SO_4$ (Zhang, Mao, Zhou, & Cai, 2005). The wet cellulose obtained are dried under the selected conditions. Figure 5 schematizes the process of obtaining regenerated cellulose.

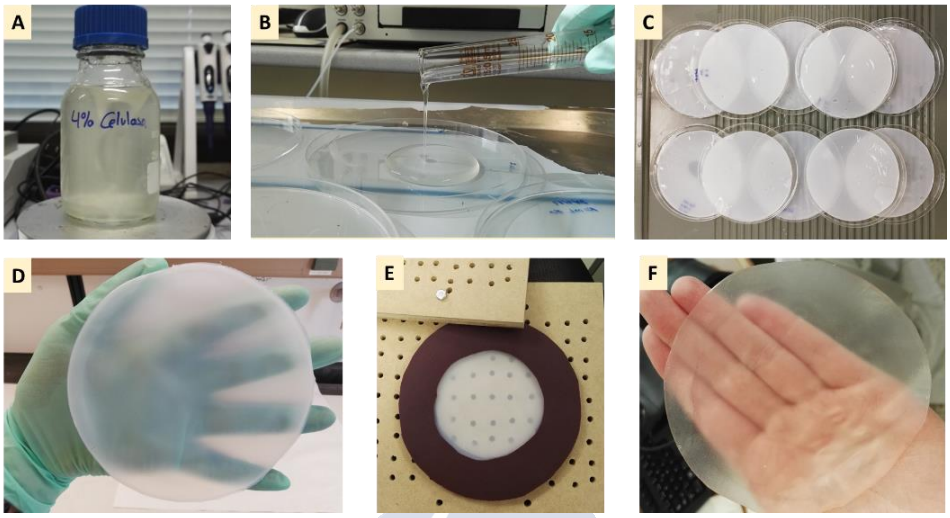

Figure 5. Process of obtaining regenerated cellulose films. A) Dissolution of microcrystalline cellulose in NaOH/urea solvent. B) A certain volume of cellulose is poured on a Petri dish. C) Cellulose films precipitated in an acetic acid bath. D) Appearance of a wet regenerated cellulose film. E) System for drying cellulose films. F) Appearance of a dry regenerated cellulose film.

Regarding bacterial cellulose, a homogeneous cellulose film can be obtained directly from the liquid culture medium. This allows the direct use of the cellulose in film form without the need for its dissolution. The 3D structure of bacterial cellulose eases combinations of cellulose with other soluble components to modify the film structure, simplifying the process, keeping intact its structure (Yang, Yao, & Wang, 2017). However, this alternative has not been extensively studied for cellulose films intended for food packaging.

On the other hand, several water soluble cellulose derivatives were developed to use cellulose as raw material and overcome the limitations

due to its water insolubility. The three hydroxyl groups present on the anhydroglucose unit can be partially or totally reacted with various reagents, resulting in cellulose esters (cellulose acetate, cellulose triacetate and cellulose acetate butyrate) and cellulose ethers (methylcellulose, hydroxypropyl methylcellulose, ethyl cellulose, hydroxyethylmethyl cellulose, carboxymethyl cellulose, and hydroxyethyl cellulose) (Bourtoom, 2008; Paunonen, 2013). Cellulose derivatives based films are generally transparent, water soluble, odorless, tasteless, flexible, with moderate strength and resistance to lipid compounds (Dhall, 2013).

Previous studies indicate that methylcellulose is the most hydrophilic water-soluble cellulose derivative and more economical (Erdohan & Turhan, 2005). Films based on hydroxypropyl cellulose and methylcellulose demonstrated efficient oxygen, carbon dioxide, and lipid barriers, but poor water vapour permeability. Studies decrease the water vapour permeability values by the addition of hydrophobic compounds, such as lipids (Villalobos, Chanona, Hernández, Gutiérrez, & Chiralt, 2005). Methylcellulose and hydroxypropylmethyl cellulose have shown interesting characteristics for application in heat treated foods, particularly in fried products, reducing the oil absorption during the frying of various foods (Varela & Fiszman, 2011).

However, the most widely known, used and commercial cellulose derivative in food packaging is cellophane. In 1908, the Swiss chemist Jacques E. Brandenberger developed the cellophane when he tried

spraying at tablecloth with a viscose solution to give it a smooth surface. He found that not only was the surface smooth but it could be peeled off like skin. Three years later he designed a machine to produce a cellophane (Robertson, 2012). During the cellophane manufacturing process, highly purified cellulose, usually derived from bleached sulfite wood pulp or cotton linters are used. First, cellulose is derivatized with carbon disulfide and sodium hydroxide to alkali-soluble sodium cellulose xanthate, called viscose. Viscose is further dissolved in dilute sodium hydroxide. The viscose liquid is extruded into a bath of sulphuric acid and sodium sulphate to reconvert it to solid cellulose. This traditional viscose route generates hazardous byproducts (CS_2 and H_2S) during manufacturing. Cellophane is insoluble in organic solvents and impermeable to fats and oils but has low water vapour barrier properties compared to synthetic polymers. It does not have the same tensile strength as plastic films and is nonresistant to strong acids and alkalis. It is flammable, hence it cannot be heat sealed. In food packaging, it is used in combination with other coatings (Bedane, Eić, Farmahini-Farahani, & Xiao, 2015; Paunonen, 2013; Robertson, 2012).

2.2. BIODEGRADABLE SYNTHETIC POLYMERS

The biodegradability properties, biocompatibility and the compatibility with natural materials of several biodegradable synthetic polymers, it is growing the strategy to combine these polymers with

polysaccharide-based films to improve the functional properties of the composite films respect to pure polymers. In addition, this strategy allows the development of new active packaging materials with properties that improve the quality, shelf life and safety of food products. Polyvinyl alcohol is one the most extended synthetic polymer used to blend with other natural materials to get composites with improved properties. Mainly, due to its water soluble property which eases the combination with other polymers in solution.

2.2.2. Polyvinyl alcohol

Polyvinyl alcohol (PVOH) is the most commercially important water-soluble plastic in use. It is an attractive polymer with many useful properties, such as low toxicity, biocompatibility, high hydrophilicity, good chemical stability, excellent film-forming proprieties, tasteless and odorless (Goodship & Jacobs, 2008; Hajji et al., 2016; Tian, Tang, Zhuang, Chen, & Jing, 2012). EFSA approved the use of PVOH as a food additive used as film coating agent for food supplements. Studies indicated that there was no evidence of toxicity, mutagenic, genotoxic or carcinogenic activity (EFSA, 2006).

PVOH is prepared by hydrolysis of polyvinyl acetate in alcohol solution. PVOH has a crystalline structure, this is unusual since it is a linear polymer with secondary alcohol groups in random positions. This is because hydroxyl groups do not interrupt the structure of the

crystalline network, due to their small size. The properties of PVOH depend on the degree of polymerization and hydrolyzation, because the presence of residual acetate groups decreases the formation of crystals (Goodship & Jacobs, 2008; Tian et al., 2012). It melts at 185 to 215ºC depending on the method of alcoholysis (Robertson, 2012).

PVOH is water soluble, slightly soluble in ethanol but insoluble in other organic solvents. For that, it can be easily mixed with several natural materials.

In the food industry, PVOH is used as a bonding and coating agent because have excellent O_2, CO_2 and N_2 barrier properties. However, it presents high water vapour permeability due to its hydrophilic nature(Goodship & Jacobs, 2008; Tian et al., 2012).

Many studies have evaluated the potential of this material to combine with biopolymers to improve mainly elastic, optical and thermal properties, such as starch, chitosan or bacterial cellulose (Frone, Nicolae, Gabor, & Panaitescu, 2015; Hajji et al., 2016; Jiang et al., 2018; Kanatt, Rao, Chawla, & Sharma, 2012; Millon & Wan, 2006; Siddaramaiah, Raj, & Somashekar, 2004).

3. PLASTICIZERS

The films obtained from several polysaccharides are brittle, usually due to interactions between polymer chains (Vieira, Da Silva, Dos Santos, & Beppu, 2011), which makes handling difficult by limiting its

applications. As consequence, the addition of plasticizers is required to obtain polysaccharides-based films.

Generally, the plasticizer acts as a lubricant, that facilitates the movement of polymer chains over each other, consequently lowering resistance to deformation (Sothornvit & Krochta, 2005). In addition, plasticizer molecules can attach along the polymer chains, replacing polymer-polymer linked and hindering the forces holding polymer chains together (Van der Waals, hydrogen bonding). This reduces the rigidity of the polymer structure, resulting in increased gel flexibility (Sothornvit & Krochta, 2005). As result, plasticizers can modify the mechanical properties of the films, reducing the tension of deformation, hardness, density and viscosity and increase the polymer chain flexibility (Vieira et al., 2011).

Some plasticizers commonly used are polyols (glycerol, sorbitol, and polyethylene glycol), sugars (glucose, sucrose), and lipids (monoglycerides, phospholipids and surfactants) (Espitia et al., 2014).

The use of polyols and sugars as plasticizers can modify the barrier properties and usually increase the water vapour permeability, due to their hydrophilic nature. In contrast, the addition of hydrophobic plasticizers could result in a decrease in water uptake (Espitia et al., 2014; Han, 2014; Vieira et al., 2011).

4. FILMS PROPERTIES

4.1. MECHANICAL PROPERTIES

In research work focus on developing new biofilms for food packaging, the analysis of the mechanical properties of the biomaterials are fundamental to know their behavior under the action of external forces. Mechanical tests help to predict their behavior and integrity during manufacturing, handling, transport and storage (Hosseini, Rezaei, Zandi, & Ghavi, 2013). Mechanical properties results will determine the purpose and potential applications of the novel biomaterial based on its viability. Comparing the data obtained of the novel polymers with the mechanical properties of commercial synthetic polymers used helps to predict the suitability and future application of the new biopolymer (Garavand, Rouhi, Razavi, Cacciotti, & Mohammadi, 2017). As well as, considering possible reformulations in the polymer composition to improve its properties and extend its suitable applications.

In the literature, the mechanical properties studies that are found are mainly the tensile test, and less often the puncture test. Generally, a texturometer is used to measure the tensile and puncture properties of biodegradable films.

Regarding the tensile test, the analysis is usually carried out following the ASTM Standard Method D882 for plastics in the form of

thin sheeting and films with less than 1.0 mm of thickness ("ASTM D882 Standard Test Method for Tensile Properties of Thin Plastic Sheeting, ASTM International," 2018). Specimens with known dimensions and thickness are placed between the grips of the texturometer fixture, as shown in Figure 6. Once the test is started, the upper arm of the texturometer separates in a vertical direction at a constant speed until the rupture of the film. The texturometer measures the applied force to keep constant the speed and the distance covered until the break of the film. The texturometer software records the data and the stress-strain graph is obtained (Figure 7). Tensile stress (TS, MPa), percentage of elongation at break (%E, %), Young's modulus (YM, MPa) and toughness (T, MJ/m^3) can be calculated from the obtained data.

TS is the maximum stress supported by the film before breaking. It represents the resistance to elongation. %E is the maximum elongation of the film before rupture. Generally, there is an inverse relationship between TS and %E of biopolymer films. The extensibility of films is reduced (lower %E) when strength of films increased (greater TS). YM is an index of the stiffness of thin plastic sheeting, obtained from the relationship between stress and strain of the polymer in the elasticity region. The T refers to the capability of a material to absorb energy and plastically deform without fracturing. Thus it is the amount of energy per unit volume that a material can absorb before rupturing (Park,

Marsh, & Rhim, 2002; Robertson, 2012; Rouhi, Razavi, & Mousavi, 2017; Spotti, Cecchini, Spotti, & Carrara, 2016).

From the tensile test, TS and %E are the most common analyzed parameters, which are compared with the values of synthetic polymers. Table 1 shows the TS and %E values of some synthetic polymers used in the food industry.

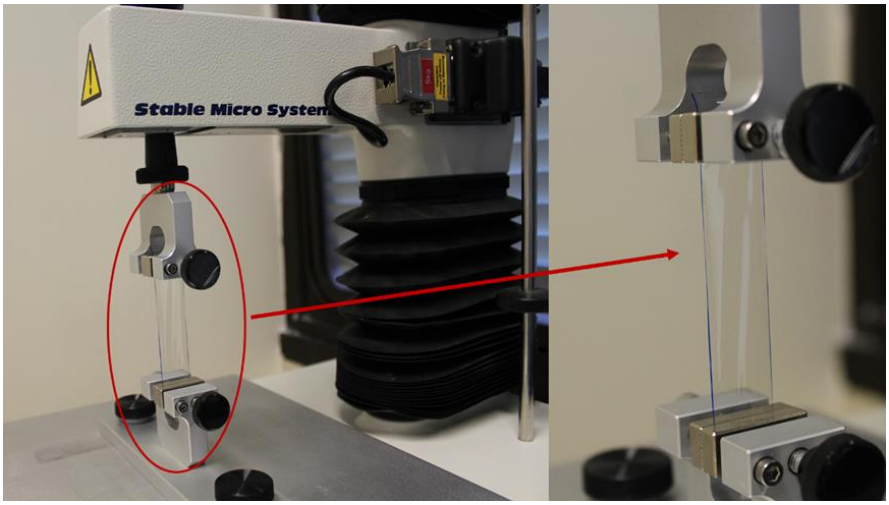

Figure 6. Sample of chitosan film fixed between the grips of the texturometer ready to run the mechanical test following the standard method ASTM D-882 (Cazon & Vázquez, 2019).

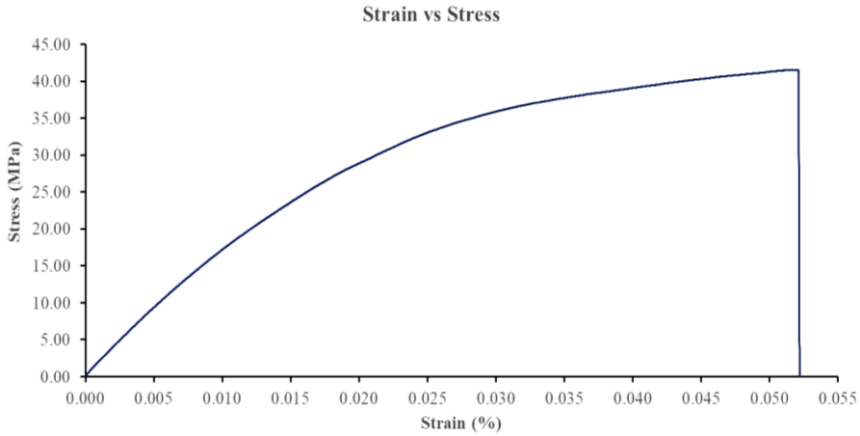

Figure 7. Example of the typical stress-strain graph obtained in the measurement of cellulose-chitosan films (Cazon & Vázquez, 2019).

Table 1. Tensile strength (MPa) and percentage of elongation at break (%) of same examples of synthetic polymers.

Film	Tensile strength (MPa)	Elongation at break (%)	Reference
High density polyethylene	29.3	859	(Su & Zhang, 2016)
Polypropylene	31 – 38	Not reported	(Pérez-Gago & Rhim, 2013)
Low density polyethylene	20	633	(AlMaadeed, Nógellová, Mičušík, Novák, & Krupa, 2014; Boldt, Gohs, Wagenknecht, & Stamm, 2016)
Cellulose acetate	1.43	Not reported	(Goetz, Jalvo, Rosal, & Mathew, 2016)

Polysaccharide-based films have shown similar TS values than synthetic polymers. However, there is an important difference in the

%E values, as shown Table 2. The values presented in Table 2 are indicative, because the mechanical properties obtained in the tensile test and puncture test depend on several factors, such as the film composition, nature of components, specimen thickness, method of preparation, measuring equipment (speed of testing, type of grips used, manner of measuring extension), previous storage conditions of the samples and measuring conditions (relative humidity and temperature). Consequently, these factors should be carefully monitored and reported if accurate comparative results are desired.

According to the reported mechanical properties of films based on cellulose and its derivatives, cellulose-based films generally showed high strength values but low elastic properties. Generally, these values depend on the composition and production method to obtain the film, such as the concentration of polymer, solvent used, molecular weight, addition of plasticizers or other components or storage conditions among others. Therefore, the studies focus on evaluating the effect of each component on the final properties of the film in order to know the interaction between the components and obtain the best formulation according to the needs. For example, it was observed that the mechanical values of methylcellulose films depend on the concentration of the polymer, showing a decrease of TS and %E values to increase the methylcellulose concentration. Values of 33±3 MPa and 14±1% were obtained using a concentration of 1.5% methylcellulose and 8±1 MPa and 6±2 % with a concentration of 6%. Authors explained

the effect of methylcellulose concentration on the mechanical properties by the partial insolubility at high concentrations. The addition of polyethylene glycol as plasticizer decreased the TS values and increased the %E values (Turhan & Şahbaz, 2004). Mechanical properties of regenerated cellulose films depend on molecular weight of cellulose used as raw material, cellulose concentration, dissolution method employed, or coagulant bath used to the regeneration of the film. Films showed TS values ranged from 60 to 90 MPa and %E ranged from 1.9 to 7.0 % (Chen et al., 2015; Xiao, Zhang, Zhang, Lu, & Zhang, 2003; Zhang, Ruan, & Zhou, 2001).

Regarding PVOH, films possess higher elasticity than cellulose-based films, but generally with lower tensile strength. The properties of PVOH films depend on several factor such PVOH concentration and degree of hydrolysis (Maria, de Carvalho, Sobral, Habitante, & Solorza-Feria, 2008). Due to the good elasticity properties of PVOH, it is widely used as plasticizer agent in polysaccharide-based films such as chitosan and starch (Bonilla, Fortunati, Atarés, Chiralt, & Kenny, 2014; Jipa et al., 2012; Srinivasa, Ramesh, Kumar, & Tharanathan, 2003; Tang & Alavi, 2011).

Table 2. Tensile strength (MPa) and percentage of elongation at break (%) of several polysaccharide-based films.

Film composition	Tensile strength MPa	Elongation (%)	References
Methylcellulose/ethanol/polyethylene glycol	25 – 33	29 – 14	(Turhan & Şahbaz, 2004)
Hydroxypropyl methylcellulose /nano-silver	51.0 - 28.3	Not reported	(De Moura, Mattoso, & Zucolotto, 2012)
Chitosan mixtures with different molecular weight and solvents	6.7 - 150.2	4.1 - 117.8	(Park et al., 2002)
Starch/plasticizers	5.06 – 68.0	2.4 - 70.7	(Muscat, Adhikari, Adhikari, & Chaudhary, 2012; Sessini, Arrieta, Kenny, & Peponi, 2016)
Tara gum/oleic acid	26.8 - 57.4	8.5 - 2.7	(Ma, Hu, & Wang, 2016)
Tara gum/chitosan	58.4 -22.7	44 – 46	(Antoniou, Liu, Majeed, & Zhong, 2015)
Brean gum/beeswax	1.6 - 7.6	8.2 - 4.9	(Spotti et al., 2016)
Pectin/nano-chitosan	58.5 - 26.1	2.9 - 0.9	(Lorevice, Otoni, de Moura, & Mattoso, 2016)
Alginate/plasticizers	18.4 - 64.7	2.5 – 7.9	(Olivas & Barbosa-Cánovas, 2008)
Agar/nano-silver	51.5 - 46.4	33.0-33.6	(Rhim, Wang, & Hong, 2013)
Chitosan/polyvinyl alcohol	13.4 – 46.9	25.5 – 116	(Hajji et al., 2016)
Polyvinyl alcohol	53.6	454	(Hajji et al., 2016)

In the effort of researchers to improve the properties of films, in recent years, nanotechnology has been incorporated into polysaccharide-based films. Generally, the purpose of the nanoparticles addition into the polymer matrix can be as reinforcing agent and/or active agent with antimicrobial or antioxidant properties among others. It was evaluated the reinforced effect of cellulose nanospheres with size of 5.9 and 10.9 nm to increase the %E of PVOH films (Ibrahim et al., 2010), reaching values up to 100%.

On other hand, puncture test determines the film strength to penetration of a cylindrical probe at constant speed (burst strength or puncture force, N/mm) and the distance at the breaking point (distance to burst or puncture deformation, mm) (Spotti et al., 2016). This test can help to predict the resistance to break of a film in contact with the protuberances of the food products, trays or boxes.

This test is also carried out with a texturometer but with a different accessory, a film support rig HDP/FSR. Figure 8 shows an example of a texturometer accessory to perform puncture tests. The samples are fixed between the support rig and submitted to perforation by a cylindrical probe, moving at constant speed until rupture. Curves of force (g) versus deformation (mm) are recorded by the texturometer software. Burst strength (BS) is calculated as the maximum puncture force until the breaking point, and distance to burst (DB) is calculated as the maximum distance of deformation at the breaking point.

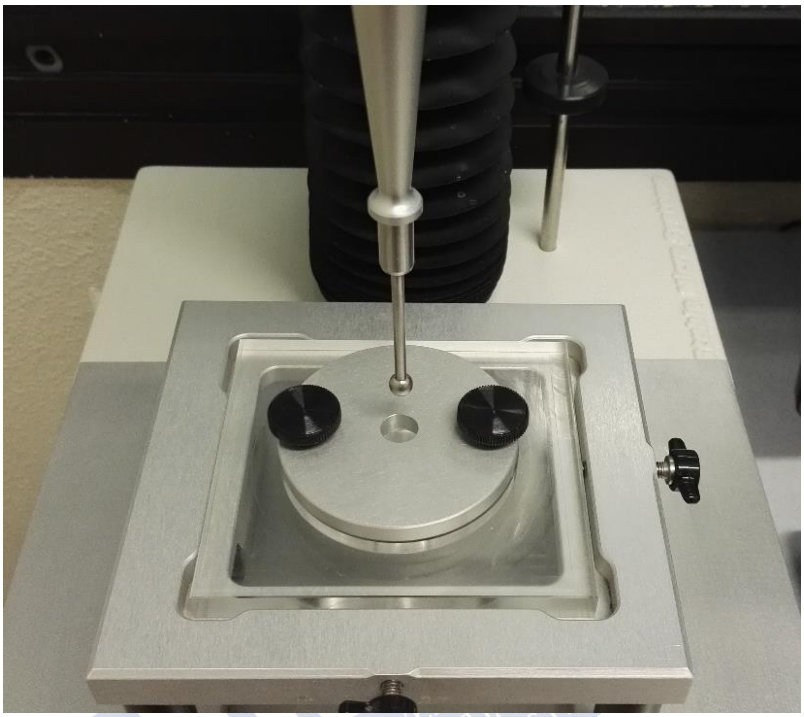

Figure 8. Example of the texturometer accessory to carry out puncture tests.

There are not many puncture data studies report in the literature. Some examples are methylcellulose-based films prepared by casting containing vegetable oil and glycerol, showed puncture strength and puncture deformation values of 147 N/mm and 3.46 mm, respectively. Nanocellulose was added to improve the puncture strength values (R. A. Khan et al., 2010). Carboxymethyl cellulose and PVOH pure film had puncture force value of 0.9 and 1.5 N, respectively. Puncture force of the composite films were higher in the 1.1–3.9 N range, as a result of the carboxymethyl cellulose-PVOH interactions (Muppalla, Kanatt, Chawla, & Sharma, 2014).

4.2. WATER VAPOUR PERMEABILITY PROPERTIES

Biodegradable polysaccharide-based films have effective barrier properties to some gases like O_2 and CO_2 (Bertuzzi, Castro Vidaurre, Armada, & Gottifredi, 2007). The efficient permeability to O_2 could help to control the ripening of fruits or reducing the oxidation of some food components like polyunsaturated fats. Moreover, the gas barrier properties of the polysaccharides-based films are important to retard loss of organic vapours, such as aroma compounds, during storage or to prevent solvent penetration in foods, which could result in toxicity or quality loss. However, due to the hydrophilic nature of polysaccharide components, these polymers own poor water vapour barrier properties (WVP) (Dutta, Tripathi, Mehrotra, & Dutta, 2009; Vieira et al., 2011).

Water vapor permeability is one of the most permeability properties studied of biodegradable films. Probably, due to the importance of water in the process of food spoilage, dehydration of fresh products or loss of crispness in dry products. It could also be due to the ease of measurement. Water vapour permeability quantifies the amount of water that permeates per unit of area and time (g/s·m·Pa), considering the pressure difference conditions under which the test is being carried out and the thickness of the film. The standard method ASTM E96 is usually the most widely used to study the water vapour

permeability by gravimetry ("Standard Test Methods for Water Vapor Transmission of Materials," n.d.).

Generally, polysaccharide-based films show higher WVP values than commercial synthetic materials. For comparative purposes, Table 3 shows WVP values of several synthetic polymers and Table 4 shows WVP of some polysaccharide-based films. As mentioned in the previous section of mechanical properties, data vary depending on the method of preparation, measuring equipment and conditions (Gennadios, Weller, & Gooding, 1994).

Table 3. Water vapour permeability (WVP) of several synthetic polymers. Modified from (Gennadios, Weller, & Gooding, 1994).

Film	WVP (g/m·s·Pa)
Polyvinylidene chloride	$0.7 \cdot 10^{-13}$ - $2.4 \cdot 10^{-13}$
High-density polyethylene	$2.4 \cdot 10^{-13}$
Polypropylene	$4.9 \cdot 10^{-13}$
Low density polyethylene	$7.3 \cdot 10^{-13}$ - $9.7 \cdot 10^{-13}$
Ethylene-vinylacetate	$2.4 \cdot 10^{-12}$ - $4.9 \cdot 10^{-12}$
Polyester	$1.2 \cdot 10^{-12}$ - $1.5 \cdot 10^{-12}$
Cellulose acetate	$0.5 \cdot 10^{-11}$ - $1.6 \cdot 10^{-11}$

Cellulose-derivative films show efficient barriers to O_2 and aroma compounds, but WVP values far from the values of synthetic polymers.

For example, methylcellulose films have WVP values ranged between $0.525 \cdot 10^{-10}$ and $0.598 \cdot 10^{-10}$ (g/s·m·Pa) (Turhan & Şahbaz, 2004). Authors justified the higher permeability due to the hydrophilic characteristic of the films with hydroxyl groups present in the polymer chain. The addition of polyethylene glycol and ethanol in the film formulation to improve the elasticity produced an increase of the WVP to increase of hydroxyl groups (Turhan & Şahbaz, 2004).

In order to decrease the WVP values, several strategies have been developed, such as the addition of lipids or nanoparticles. WVP properties of hydroxypropyl methylcellulose/whey protein films were improved by adding lipids. However, it resulted in an opaque, unstable, brittle, and waxy taste film (Rubilar, Zúñiga, Osorio, & Pedreschi, 2015). Other samples is methylcellulose and hydroxypropylmethyl cellulose films with stearic/palmitic fatty acids and beeswax forming a surface coat (Kester & Fennema, 1989) showed low WVP values ($6.61 \cdot 10^{-13}$ g/s·m·Pa). WVP in the same order of magnitude as synthetic polymers. These wax-laminated films are potentially useful in any food product where undesirable intercomponent moisture migration takes place (Kester & Fennema, 1989).

On the other hand, studies indicate that nanoclays or another nanoparticle can modified the permeability properties of polysaccharides-based films (Chivrac, Pollet, & Avérous, 2009).

Table 4. Water vapor permeability (WVP) of several polysaccharide-based films.

Film composition	WVP (g/m s Pa)	References
Methylcellulose/hydroxypropyl methylcellulose with plasticizers and lipids)	$6.61 \cdot 10^{-13}$ - $1.63 \cdot 10^{-11}$	(Kester & Fennema, 1989)
Methylcellulose/ethanol	$0.51 \cdot 10^{-10}$ - $1.08 \cdot 10^{-10}$	(Turhan & Şahbaz, 2004)
Methylcellulose/nanocellulose	$5.44 \cdot 10^{-11}$ - $7.29 \cdot 10^{-11}$	(Khan et al., 2010)
Hydroxypopyl methylcellulose/nano-silver	$1.54 \cdot 10^{-10}$ - $2.22 \cdot 10^{-10}$	(De Moura et al., 2012)
Agar/nano-silver	$1.67 \cdot 10^{-9}$ - $1.97 \cdot 10^{-9}$	(Rhim et al., 2013)
Chitosan/starch	$1.18 \cdot 10^{-10}$ - $1.55 \cdot 10^{-10}$	(Santacruz, Rivadeneira, & Castro, 2015)
Chitosan	$0.32 \cdot 10^{-9}$ - $0.51 \cdot 10^{-9}$	(Park et al., 2002)
Chitosan	$1.77 \cdot 10^{-9}$ - $2.26 \cdot 10^{-8}$	(Kim, Son, Kim, Weller, & Hanna, 2006)
Chitosan-tapioca starch	$2.8 \cdot 10^{-10}$ - $6.7 \cdot 10^{-10}$	(Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009)
Tapioca starch	$12.1 \cdot 10^{-10}$	(Vásconez et al., 2009)
Cassava starch/glycerol	$1.8 \cdot 10^{-10}$ - $2.8 \cdot 10^{-10}$	(Seligra, Medina Jaramillo, Famá, & Goyanes, 2016)
Tara gum/plasticizers	$0.52 \cdot 10^{-10}$ - $0.69 \cdot 10^{-10}$	(Ma et al., 2016)
Alginate/plasticizers	$6.5 \cdot 10^{-10}$ - $0.93 \cdot 10^{-9}$	(Olivas & Barbosa-Cánovas, 2008)
Agar/nano-silver	$1.67 \cdot 10^{-9}$ - $1.97 \cdot 10^{-9}$	(Rhim et al., 2013)
Quinoa/chitosan	$2.61 \cdot 10^{-10}$	(Abugoch, Tapia, Villamán, Yazdani-Pedram, & Díaz-Dosque, 2011)

Moreover, polysaccharides are used as raw material to prepare nanocomponents to reinforce the structure of another polymer matrix. The most common polysaccharides used for nanoparticles production in biodegradable films are cellulose, starch and chitosan (Mondal, 2017; Wahyuningsih et al., 2016). Some examples are methylcellulose with nanocellulose (Khan et al., 2010), tara gum film with cellulose nanocrystals (Ma et al., 2016) and gelatin nanocomposite films containing bacterial cellulose nanocrystals (George & Siddaramaiah, 2012). However, in these cases, results did not show a significant change in the WVP with the addition of polysaccharide nanoparticles. Unlike, PVOH films with different amounts of cellulose nanocrystals as filler, using poly(acrylic acid) as a crosslinking where the cellulose nanocrystals have a significant effect on WVP, decreasing the permeability (Paralikar, Simonsen, & Lombardi, 2008).

Despite the poor water vapour barriers of polysaccharide-based films, they can have potential applications on food. Since they are generally highly hygroscopic, polysaccharide-based films can be applied as relatively thick films on the surface of food to absorb water. In this way, the films can provide temporary protection against moisture loss of the food until the coating becomes dehydrated (Bourtoom, 2008; Embuscado & Kerry, 2009).

4.3. OPTICAL PROPERTIES

UV-light (200-400 nm) can have negative effects when indexed directly on food. UV radiations can generate the appearance of free radicals in the products as result of several organic photochemical reactions. Singlet oxygen, produced by UV radiation from sunlight, is the fastest cause of oxidation of lipids, antioxidants, vitamins and proteins. These reactions are responsible for alterations in the flavor, taste, color and lost nutritional quality of food. This leads to a loss of the organoleptic properties of the product that make it less palatable or unacceptable to the consumer, reducing its shelf life (Goudarzi, Shahabi-Ghahfarrokhi, & Babaei-Ghazvini, 2017; Guo, Ge, Li, Mu, & Li, 2014). Hence, developing polysaccharide-based films to food packaging with good UV-light barrier properties can help to slow the oxidation process of the food components, increasing the shelf life of the food product keeping the food quality and adequate organoleptic properties (Cazon & Vázquez, 2019).

The evaluation of the barrier properties against UV radiation is not a commonly evaluated property, despite the important effect it could have on food packaging. Some examples reported is starch-based films with TiO_2 as a protective agent against UV radiation (Goudarzi et al., 2017), chitosan-based films (Ahmed & Ikram, 2016; Vilela et al., 2017), PVOH-based films with bio-oil components (Morales, Andrés, Labidi, & Gullón, 2019) or starch/kefiran/ZnO (Babaei-Ghazvini,

Shahabi-Ghahfarrokhi, & Goudarzi, 2018) composite films among others.

In addition to the UV barrier properties, other important optical properties that must be taken into account when developing new biodegradable films are the color and transparency of the film. Color and transparency have a direct impact on the consumer acceptability (Ketelsen et al., 2020). Consumers prefer transparent packaging to be able to see the packed product and to observe the visual quality (Vilela et al., 2017). Therefore, it is important to formulate films with good protective barriers against UV radiation while maintaining adequate color and transparency properties.

4.4. MOISTURE ADSORPTION ISOTHERMS

Polysaccharide-based films are hydrophilic materials that can interact with the environmental moisture. Therefore, the water content of the films depends largely on the surrounding humidity and temperature. Generally, the water molecules adsorbed by the biopolymer exert a plasticizing effect. The variation in the moisture content of the film can promote structural changes in the organization of the polymer chains, resulting in modifications in the mechanical, barrier or optical properties of the film, among others due to the plasticizing effect (Sothornvit & Krochta, 2005). Accordingly, the evaluation the interaction of the polymer with the environmental

humidity, as well as the water effect on its functional properties is important in order to predict the stability and quality changes that could occur during the packaging and storage of the food product (Srinivasa, Ramesh, & Tharanathan, 2007).

The analysis of moisture adsorption isotherms allows evaluating the water activity (a_w) of the film at several equilibrium relative humidity conditions (RH_{eq}). The relationship between a_w and RH_{eq} is established when equilibrium adsorption is achieved by the samples stored in hermetically sealed microclimates at certain temperature and humidity conditions. During the conditioning of the samples at a certain temperature and humidity, a dynamic process takes place in which the water molecules leave the surface of the sample (desorption), while other molecules adhere to the surface (adsorption). A dynamic equilibrium state is reached and the number of molecules that leave the surface and those that adhere to the surface is the same with the necessary storage time of the samples in closed microclimates. Thus, the number of molecules on the surface of the film remains constant on average. Hence, the relationship between the a_w and RH_{eq} is established because the a_w of the sample becomes equal to the RH_{eq} of the surrounding atmosphere (Figura & Teixeira, 2007; Lewicki, 1997).

Mathematical models are used to calculate the adsorption isotherms in order to know the relationship between a_w and moisture content of the sample in a wide spectrum of RH_{eq}. Initially, the mathematical model Brunauer, Emmett and Teller (BET) was the most

widespread method to estimate the water sorption in food products (Timmermann, Chirife, & Iglesias, 2001). However, BET model is applicable only in range of a_w up to 0.5. Two familiar parameters are obtained from the BET model, the monolayer moisture content (X_m) and the energy constant (C). Despite the theoretical limitations of the BET model, the BET monolayer concept is an useful parameter for several aspects of interest in food products and edible films (Lewicki, 1997; Timmermann et al., 2001). The BET equation has become a simple and resorted method to estimate the moisture mass of the monolayer on the surface of the materials (Figura & Teixeira, 2007).

Recently, other mathematical model was developed to describe the moisture sorption behavior of food products or edible films, being the isothermal equation of Guggenheim, Anderson and Boer (GAB). Unlike the BET model, the GAB equation allows representing the experimental data in a wide range of a_w, up to 0.9 (Lewicki, 1997; Timmermann et al., 2001). For this reason, the GAB model was substituting the BET model and has been recommended by the European Project Group COST 90 as the fundamental equation to evaluate the moisture sorption in food materials (Wolf, Spiess, & Jung, 1985).

In the GAB model, two parameters appear apart from the moisture content in the monolayer. The constant C related to the strength of the water bindings to the primary adsorption sites, and K that refers to the heat of the multilayer sorption (Bedane et al., 2015). Therefore, the

GAB model introduces a second stage of sorption for water molecules (Timmermann et al., 2001).

The shape of the sorption isotherm obtained following the GAB model can help to predict the water molecules bonding states as a function of water activity. Generally, polysaccharide water sorption isotherms have a sigmoidal shape with three regions (Figure 9) (Figura & Teixeira, 2007; Lewicki, 1997).

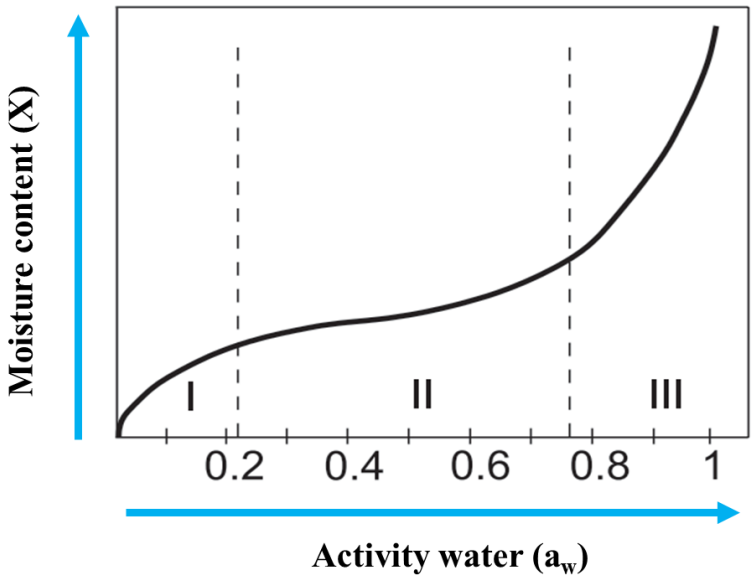

Figure 9. Generic sorption isotherm showing main regions of water bonding. Modified from (Figura & Teixeira, 2007).

Region I corresponds to the region with the lowest aw and moisture content. In this region, water molecules are strongly bound with polymer free active groups. These water molecules are not available to

contribute any kind of moisture behavior to the material, do not freeze and form a monolayer.

Region II is where the monolayer is completely saturated. Closer layers of water molecules are formed in which the particles are not completely free. They form the so-called partially bound water that can be freezing. In region III, there is free water that is not linked and is filling the pores of the capillary. It is water that freezes and can be removed in a centrifugation process (Ludwiczak & Mucha, 2010).

Previous research works have studied the relationship between moisture content and a_w of polysaccharide-based films, such as cellulose (Belbekhouche et al., 2011; Meriçer, Minelli, Giacinti Baschetti, & Lindström, 2017; Zografi, Kontny, Yang, & Brenner, 1984), several gums (Pérez-Alonso, Beristain, Lobato-Calleros, Rodríguez-Huezo, & Vernon-Carter, 2006), carboxymethyl chitosan (Tantala, Rachtanapun, Tongdeesoontorn, Jantanasakulwong, & Rachtanapun, 2019), chitosan (Aguirre-Loredo, Rodríguez-Hernández, Morales-Sánchez, Gómez-Aldapa, & Velazquez, 2016; Ludwiczak & Mucha, 2010; Monte, Moreno, Senna, Arrieche, & Pinto, 2018), κ-carrageenan (Popescu, Dogaru, Sun, Stoleru, & Simionescu, 2019) or starch (Bertuzzi, Armada, & Gottifredi, 2007; Javier I. Enrione, Sandra E. Hill, & Mitchell, 2007; Mali, Sakanaka, Yamashita, & Grossmann, 2005) among others.

5. APPLICATION

Biodegradable films from polysaccharides or blend of polysaccharides with several compounds including other polysaccharides, proteins, lipids, additives or biodegradable polymers, have been developed and evaluated to extend the shelf-life and preserve the quality and safety of several foods (Galus & Kadzińska, 2015). The research works has demonstrated the extensive applications of these biodegradable components in food packaging, including new trends such as active and/or intelligent packaging.

Reviewing the literature, there are several research papers on the application of cellulose-based films or PVOH films combined with other components to increase the shelf-life and food safety of foods such as meat, fruit or vegetables, maintaining their quality and properties.

Hydrophobic coatings based on cellulose nanofibers were developed and validated for reducing cherry rain-cracking, resulting in a significant reduction in cherry rain-cracking (31.18–44.60%), with no negative effect on fruit firmness, size, soluble sugar, pedicel/fruit retention force or color compared to uncoated cherries (Jung, Deng, Simonsen, Bastías, & Zhao, 2016). A subsequent study evaluated consumer acceptance and quality of processed blueberries and cherries subjected to the previously developed cellulose nanofiber coating. The consumer acceptance study showed that the coating improved the

texture and taste scores of the aroma of blueberries, and increased the taste for the aroma of cherries compared to uncoated fruits (Jung, Simonsen, Wang, & Zhao, 2018).

A nanocellulose-based coating with oleic acid and sucrose ester fatty acid was developed to control post-harvest physiological activity and increase storage. This coating was tested on bananas stored at room temperature. The coating delayed the ethylene biosynthesis pathway, reducing the production of ethylene and CO_2, which delayed fruit ripening. This way, the coating minimized the degradation of chlorophyll, weight loss and firmness of bananas while ensuring proper ripening of the fruit for 10 days of storage at room temperature (Deng, Jung, Simonsen, & Zhao, 2017). Similar results were observed in nanocellulose coatings combined with chitosan and oleic acid for coatings on Bartlett pears during long-term cold storage (Deng, Jung, Simonsen, & Zhao, 2018) and for methylcellulose-stearic acid coatings to preserve the quality of Anjou pear wedges (Olivas, Rodriguez, & Barbpsa-Cánovas, 2003). The commercially developed cellulose-based coating, called NatureSeal®, was studied as a transporter of antioxidants, acidulants and preservatives to prolong the shelf-life of freshly cut apples when stored in wrapped trays during 1 week at 4 °C (Olivas et al., 2003).

Other examples of cellulose-based film or coating applications are its evaluation in muscle foods to reduce oil absorption during frying minimize runoff during cooking and reduce moisture loss when applied

as glazes for poultry and shellfish. Also its application as an oxygen and oil barrier and oil for food such as pizza and ice cream cones (Cutter, 2006).

Regarding examples of PVOH-based films applied on food products, composite bilayer film based on polyvinyl alcohol, sodium alginate and chitosan were evaluated as coating of salted duck eggs during storage. The coatings significantly improved the deterioration of the quality of salted duck eggs, such as water loss, oil exudation, texture and egg yolk pH. The double layer coating allowed to prolong the shelf-life of the eggs more than 60 days at 25 °C and 50% RH according to the results of the total bacterial count experiment, while the shelf-life of the control eggs was less than 45 days (Jiang et al., 2018).

In recent years, food packaging with antimicrobial properties have attracted a lot of attention due to the consumer demand for minimally processed and synthetic preservative-free products. The tendency is to develop materials, such as films or coatings, with the antimicrobial agent incorporated. The direct contact of the antimicrobial film or coating allow transfer only low levels of preservatives into the food. In addition, the tendency to use natural antimicrobial components that inhibit the growth of common foodborne microorganisms increases (Cha & Chinnan, 2004). Thereby, bacterial cellulose-based films combined with nisin have been evaluated to inhibit *Listeria monocytogenes* in processed meats, studying the effectiveness on the surface of packaged frankfurter sausages. The results showed a

significant decrease in the *L. monocytogenes* count in sausages of ~ 2 log $CFUg^{-1}$ after 14 days of storage compared to control sausages (Nguyen et al., 2008). The viability of a bacteriostatic sausage casing made of bacterial cellulose embedded in ε-polylysine was also evaluated. The novel sausage causing exhibited bacteriostatic activities against a broad spectrum of Gram-positive and Gram-negative bacteria, extending the shelf-life the sausage packaged with the developed composite film than controls (Zhu et al., 2010).

Another widely used strategy is the use of essential oils of natural components as antimicrobial agents and even with antioxidant properties (Yuan, Chen, & Li, 2016). Carboxymethyl cellulose-polyvinyl alcohol blend films with clove oil were applied in ground chicken meat. Meat samples packed with the active film had lower total viable counts than unpacked control samples. The shelf-life of the product was extended until to 12 days, while the control samples spoiled within 4 days during refrigerated storage. The efficacy of these films was also demonstrated in studies of packed inoculum against *Staphylococcus aureus* and *Bacillus cereus* in ground chicken meat (Muppalla et al., 2014).

New technologies developed in recent years, such as 3D printing, can provide new opportunities for the use of cellulosic materials such as food packaging. This topic has attracted a lot of interest from the research community in the last decade, and much work has been done in the application of cellulosic materials in 3D printing. Potential

materials include cellulose ethers/esters, microcrystalline cellulose, nanocellulosic materials and other products (Dai et al., 2019).

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CHAPTER II - OBJECTIVES

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GENERAL OBJECTIVE

To develop and characterize novel functional cellulose-based films with improved properties by the combination with polyvinyl alcohol and glycerol, using regenerated or microbial cellulose.

SPECIFICS AIMS

To evaluate the effect of polyvinyl alcohol and glycerol on the functional properties and structure of films based on regenerated cellulose.

To analyze the effect of moisture content on the properties of films developed based on regenerated cellulose-polyvinyl alcohol-glycerol.

To evaluate the effect of polyvinyl alcohol and glycerol on the functional properties and structure of films based on bacterial cellulose.

To analyze the effect of moisture content on the properties of films developed based on bacterial cellulose-polyvinyl alcohol-glycerol.

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CHAPTER III – RESULTS AND DISCUSSION

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CHAPTER III - RESULTS AND DISCUSSION

1. REGENERATED CELLULOSE - GLYCEROL - POLYVINYL ALCOHOL COMPOSITE FILMS FOR FOOD PACKAGING: CHARACTERIZATION

The purpose of this study was the characterization of regenerated cellulose-based films combined with glycerol and PVOH. Polynomial models were obtained to describe the effect of the mixture on mechanical, permeability and water adsorption properties. Besides, the morphological, optical, structural and thermal properties of the films were analysed by scanning electron microscopy (SEM), UV-VIS spectrophotometer, Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC).

Composite films were prepared using microcrystalline cellulose, glycerol and PVOH at several concentrations following the experimental design showed in Table 1 of Annex 1. Certain amount of microcrystalline cellulose was dispersed in an aqueous solution of NaOH:urea:H_2O (7:14:79 w/w). Different solutions were prepared with final cellulose concentrations ranging 3-4% (w/w). Glycerol is directly added into the transparent cellulose suspension to reach a final glycerol concentration ranging 0-5% (w/w). Please note glycerol concentration higher than 5% (w/w) destabilized cellulose solution. Regenerated cellulose films were obtained from the cellulose-glycerol solution

following the coagulation process by immersion in acid bath. Next, the resulted regenerated cellulose films were immersed in PVOH baths with a concentration ranging 2-10% to obtain the final composite film based on regenerated cellulose-glycerol-PVOH. Pure regenerated cellulose films were brittle and during the drying process films broke easily. For that, a minimum concentration of 2% (w/w) PVOH was stablished to avoid ruptures during the drying process, ranged the PVOH concentration from 2 to 10% (w/w). Finally, the film was dried in a Petri dish at room temperature for 2 days. The dried samples were cut to a specific size and stored in desiccators for 5 days at 57 or 0 % RH to carry out each experiment. The filmmaking process is explained in detail in "Materials and methods" section of the published articles of Annex 1 and Annex 2.

Results of dependent variables TS, %E, T, BS, DB, WVP and %W are shown in Table 1 of Annex 1 and Annex 2. The set of experiments followed the Box–Behnken design which is formed by combining two level factorial designs with incomplete block designs. The statistical analysis was performed using Design Expert® 10.0.6 software. Cook´s distance was applied the detect outliers. The models were validated through multifactor analysis of variance (ANOVA) test. Tables 2 of Annex 1 and Annex 2 show the ANOVA results for each dependent variable.

Tensile test was carried out using texturometer (TA-XTplus, Stable Micro System, UK) following the standard method D-882 (ASTM). In

the texturometer, the 4 mm/s of crosshead speed and 40 mm initial grip separation were set up. Ten specimens of dimensions 15×100 mm, previously conditioned in desiccators at 57% RH for 5 days, were measured for each formulation. TS, %E and T were measured with the texturometer. Tensile test results showed TS values ranged from 25.90 to 368.82 MPa. TS value decreased to increase cellulose concentration due to the increase of cellulose crystallinity, which promoted the brittleness of the film. The incorporation of PVOH induced the interaction between components and reinforced the matrix, enhancing the resistance to break. The effect of glycerol depended of the concentration of cellulose and PVOH, and their interactions.

The %E values obtained were in the range 0.89-18.74%. Results indicated that %E was highly affected by the concentration of PVOH and the interaction glycerol-PVOH, following by the interaction cellulose-PVOH. The interaction cellulose-glycerol had no significant effect on %E. PVOH acted as plasticizer on cellulose matrix, forming new intermolecular hydrogen bonds between cellulose-PVOH, weakening the hydrogen bonds among cellulose polymeric chains. The increase of cellulose concentration increased slightly the PVOH effect on elasticity. However, glycerol manifested an anti-plasticizing effect in the cellulose matrix, decreasing the E% values to increase its concentration. A strong interaction might be taking place between the polymer and the small quantity of plasticizer resulting in a “cross-linking” effect, which decreases the free volume and mobility of the

chains. Besides, small amount of plasticizer could promote the reorganization of the chains increasing the structure crystallinity.

T values were in the range of 0.25-44.30 MJ/m^3. T values increased by increasing PVOH content, in response to the reinforcing and plasticizing effect that PVOH showed in regenerated cellulose films. The interactions between cellulose-PVOH allowed the sliding of the polymer chains during the mechanical test, improving the capability to absorb energy and plastically deform without fracturing. However, glycerol showed a negative effect on toughness values, decreasing T to increase glycerol content, similar behaviour showed in elasticity properties. The interaction cellulose-glycerol reduced the direct interaction among cellulose chains, reducing the molecular mobility and toughness properties.

Puncture test was carried out using a texturometer (TA-XTplus, Stable Micro System, UK) with a film support rig (HDP/FSR). A compressed speed of 60 mm/min was set up. Four squares of each film of 30 mm side were measured for each film formulation, previously stored in desiccators at 57 %RH for 5 days. BS and DB were measured in this texturometer mode.

Results of puncture test showed values of BS in the range from 1395 to 12014 g and DB in the range from 1.60 to 6.04 mm. In both cases, the linear effect of PVOH concentration is the only significant term. This behaviour is probably due to the plasticizing and reinforcing effect of PVOH into the cellulose matrix, that eased the reorganization

of the polymer chains during the puncture deformation, improving the puncture resistance and elasticity of the blend. Besides, the increase of PVOH concentration produced the increase of the film thickness. Unlike the properties measured in the tensile test, the puncture test does not take into account the thickness of the film that affects its puncture resistance.

WVP were measured following the gravimetrically method established by the ASTM Standard Test Method E96 at 20°C inside a desiccator with silica gel. Weight of the cup was recorded during 5 days to the nearest $1 \cdot 10^{-4}$ g and plotted as a function of time. Films showed values of WVP in the range $2.32 \cdot 10^{-11}$-$3.01 \cdot 10^{-11}$ g/s·m·Pa. WVP values increased as the glycerol concentration increased, as result of the hydrophilic nature of glycerol. WVP decreased slightly by increasing of cellulose concentration, resulting in higher values at lower cellulose concentration. Cellulose has high crystalline regions, inaccessible to water molecules, having only access to the paracrystalline regions. The increase of cellulose content could increase the crystallinity region of the film, hindering the water vapour diffusion. Higher values of WVP were obtained at intermediate concentrations of PVOH. The WVP values obtained from the developed films in this study are 2 orders of magnitude higher from the WVP values from synthetic polymers. However, the cellulose-PVOH composite films showed lower WVP values than pure PVOH films.

The %W was determined by gravimetric method, measuring the difference in weight between samples immersed in distilled water for 24 h and its dry weight. %W values calculated ranged from 136.35 to 222.09%. PVOH produced an increase of the %W values, with no significant variations in the %W depending on cellulose and glycerol content.

Predictive mathematical models were obtained to determine the mechanical, WVP and %W properties of the developed films. The predictive models allow selecting the correct formulation to obtain films with any value in the study range, according to the needs. The predictive mathematical models and the response surface graphs of the effect of each independent variable on the dependent variables can be found in Annex 1 and Annex 2.

The optical properties of regenerated cellulose-glycerol-PVOH composite films were measured using a spectrophotometer V-670 (Jasco Inc, Japan) in transmittance mode in UV-VIS-NIR regions. The transparency of the films was calculated from the percent transmittance value at 600 nm. Results indicated the good barrier properties in the UV-light range (200-280 nm) of regenerated cellulose-glycerol-PVOH samples. Glycerol showed UV-protective properties, decreasing the light transmittance of the samples to increase the glycerol content. Unlike PVOH effect on UV-light region, samples showed a very low adsorption in the UV region to increase the PVOH content up to 10%.

However, the presence of PVOH improved the transparency and visual appearance of cellulose film.

The interaction between the components were analysed by SEM images, FTIR and DSC. SEM imaged showed that PVOH softened the surface of the film, decreasing the roughness and porosity of the samples. FTIR and DSC showed the interaction between the components of the films and confirms that glycerol was retained after the film regeneration process. Results indicated that glycerol and PVOH increased thermal stability of the films, which is attributable to the increased interaction between the plasticizers and cellulose.

Justification, material, methodology, results and discussion of this section are extensively detailed in **Annex 1 and Annex 2.**

2. BACTERIAL CELLULOSE - GLYCEROL - POLYVINYL ALCOHOL COMPOSITE FILMS FOR FOOD PACKAGING: CHARACTERIZATION

This work deals with the characterization of BC-glycerol-PVOH composite films. Mathematical models were obtained to assess the effect of PVOH and glycerol on moisture content (%), TS, %E, Young's modulus (YM), T, BS, DB, WVP, %W and solubility (%S) properties of cellulose-based composite films. Optical properties, microstructure, structure and thermal properties of the blends were analyzed by UV-VIS spectrophotometry, SEM, FT-IR and thermogravimetric and differential scanning calorimetry (TGA-DSC).

Composite blend films were prepared using BC films combined with glycerol and PVOH at several concentrations, following the experimental design showed in Table 1 of Annex 3 and Annex 4.

The first step was to obtain pure bacterial cellulose films. Culture medium containing 10% (w/w) glucose and 1% (w/w) yeast extract was used to grow *K. xylinus* and produces BC. The fermentation conditions were at 30 ºC for 8 days under static state. After this time, a homogeneous film suspended on the surface of the liquid medium was formed and removed. BC film was treated with NaOH and water to remove possible remains. The wet BC film was at room temperature for 48 h, obtaining pure BC film.

BC composites were obtained from the resulted wet bacterial films by dipping in mixed baths of glycerol and PVOH ranged from 0 to 5% (w/w). Resulted pellicles were dried in a Petri dish at room temperature for 48 h.

The dried samples were cut to specific sizes for each test and stored in desiccators at 57%RH or silica gel for 5 days, ready to measure. The process of BC-glycerol-PVOH samples production is extensively described in “Materials and methods” section of the Annex 3.

Results obtained of each dependent variable moisture content (%), TS, %E, YM, T, BS, DB, WVP, %W or %S are shown in Table 1 of Annex 3 and Annex 4. The set of experiments followed the Box–Behnken design, combining two level factorial designs with incomplete block designs. Design Expert® 10.0.6 software was used to perform the

statistical analysis. Cook´s distance was applied the detect outliers. The models were validated through multifactor analysis of variance (ANOVA) test. Tables 2 and Table 3 of Annex 3 and Annex 4 show the ANOVA results and the fit statistics values for each dependent variable.

Equilibrium moisture content of the samples at 57% RH was determined by gravimetric method. Glycerol and PVOH have a strong hydrophilic nature, with strong interaction with water of environmental humidity. Under standard conditions, the moisture contents between the samples may vary due to their formulation. Water has a plasticizing effect on polysaccharide-based films. This way, the analysis of moisture content of each sample under standard conditions can help to understand the results of the properties studies, such as mechanical properties. The moisture content of the samples stored in desiccators with saturated solution of sodium bromide for 5 days ranged from 1.82 to 91.10 %. Glycerol showed higher effect on the moisture content properties than PVOH, increasing significantly the moisture content of the specimens. The hygroscopicity and hydrophilic nature of glycerol resulted in an increase of the moisture content from 1.82% for pure BC samples, up to 91.10% for BC-GLY (5% w/w).

TS, %E and YM were calculated from the tensile test, using a texturometer (TA-XTplus, Stable Micro System, UK) following the standard method D-882 (ASTM). The equipment configuration was a crosshead speed of 0.08 mm/s and 40 mm of initial grip separation. For each run, ten samples of 15×100 mm were measured. Previously to

carry out the test, the samples were stored in a desiccator at 57% RH for 5 days. The mechanical parameters were calculated from the stress-strain curves obtained. TS values ranged from 1.23 to 35.05 MPa, %E values ranged from 2.28 to 49.89%, YM values ranged from 10.84 to 1489.15 MPa and T values ranged from 0.22 to 2.60 MJ/m^3. Results indicated that the addition of PVOH showed a reinforcing effect on the BC matrix meanwhile glycerol manifested a significant plasticizing behavior. The presence of PVOH resulted in a denser structure, yielding more resistant films. Glycerol acted as internal lubricant, promoting the mobility of the polymeric chains by reducing friction forces between cellulose ribbons. Besides, glycerol produced an increase of the water content of the samples, which also had a plasticizing effect.

On the other hand, puncture test was carried out using the same texturometer with a film support rig (HDP/FSR) and a compressed speed of 60 mm/min. Four squares of each film of 30 mm side were measured for each film formulation. BS values obtained ranged between 43.74 and 2105.52 g and DB ranged from 0.39 to 4.94 mm. PVOH and glycerol showed similar effects than those observed in tensile tests. The improvement of puncture properties was due to the packed structure of BC-PVOH composites films resulted in less porosity and thicker structure than pure BC. Besides, both polymers are capable of forming strong, intra- and intermolecular hydrogen bonds between hydroxyl group. Glycerol produced important changes in the elasticity of the film. Glycerol eased the mobility of the polymeric

chains by reducing friction forces between cellulose fibers. The simultaneous addition of PVOH and glycerol to BC films resulted in a significant increase of the elasticity, due to the combination of the reinforcing and plasticizer effect of each component.

WVP were measured following the gravimetrically method established by the ASTM Standard Test Method E96. In this case, the test was carried out at 30°C using a wide-mouth cup filled with distilled water and double-wall box containing silica gel placed on the plate of a precision balance. The test was run for at least 8 h, enough time to reach a dynamic equilibrium in the water flux. WVP values ranged from $1.87 \cdot 10^{-11}$ to $2.04 \cdot 10^{-10}$ g/m s Pa. The combination of BC with PVOH resulted in a slightly decrease of the WVP of cellulose samples. Although PVOH is a hydrophilic polymer, it reduced the porosity of the film giving a closed-packing structure that hindered the water vapour diffusion. However, the presence of glycerol, due to its hydrophilic nature, increased the values of the WVP. Besides, the SEM images of BC-glycerol samples showed larger inner free volume and higher porosity than pure BC. The internal modifications eased the diffusion of water molecules through the matrix, increasing the WVP.

The %S of the samples were measured following the gravimetric method by the difference in the weights of dry samples before and after being immersed in distilled water under stirring for 24 h at 25ºC. The values obtained ranged from 9.37 to 31.65%. Glycerol and PVOH are water-soluble, hence part of the PVOH and glycerol were dissolved in

water, reaching solubility values up to 31.65%. However, in presence of both glycerol and PVOH components at higher concentrations, %S decreased to 13.23%. BC-GLY-PVOH interactions promoted the retention of the components into the cellulose matrix, being stronger at the highest PVOH and glycerol concentrations.

The %W was measured following the gravimetric method, by weight difference between dry and wet samples. %W values obtained ranged 78.26-364.78%. The high porosity, three-dimensional structure and bigger surface contact of pure BC films eased the retention of water inside the matrix. %W was radically decreased by adding glycerol and PVOH. The effect of glycerol was more significant than PVOH effect. Probably, glycerol was easier to diffuse into the film, producing a greater coverage of cellulose fibers that limited the retention of water molecules.

Mathematical models were obtained to predict the moisture content, mechanical, WVP, %S and %W of the developed films. The predictive models allow selecting the correct formulation to obtain films with any value in the study range, according to the needs. The predictive mathematical models and the response surface graphs of the effect of each independent variable on the dependent variables can be found in Annex 3 and Annex 4.

The optical properties were analyzed using a spectrophotometer V-670 (Jasco Inc, Japan) in transmittance mode. Tables 4 of Annex 3 and Annex 4 summarize the average percentage of transmittance in the UV-

A, UV-B and UV-C regions, transparency and opacity values of the samples. Pure BC films showed good UV-barrier properties, with low transmittance values in the UV-C region (1%). Glycerol improved the UV-light barrier properties, showing transmittance values of 0.57% of BC-glycerol samples. The presence of PVOH increased the transmittance values of the samples, resulted in an increase of the transparency values and decrease of opacity values.

The interaction between the components were analysed by SEM images, FTIR and DSC. The SEM images of the samples showed that BC matrix consisted of slender fibrils, 30 to 40 nm wide, lying in various directions and assembled forming porous. The films showed laminated structure. PVOH and glycerol penetrated through the fibers to the inner layers of the BC. PVOH penetrated promoted a more compact, denser and thicker film. Besides, PVOH coated the surface of the film, resulting in a softening of the surface, diminishing the roughness.

FT-IR and TGA-DSC evidenced the interaction among the polymers. FT-IR analysis indicated that the presence of PVOH and glycerol caused the increase in adsorption peaks in the major wavelength regions associated with hydroxyl groups. Thermal analysis suggested that part of the glycerol and PVOH of the composites were degraded at high temperatures due to the BC-glycerol-PVOH interactions, enhancing the thermal stability of the composite films and increasing the temperature of their thermal degradation.

Justification, material, methodology, results and discussion of this section are extensively detailed in **Annex 3 and Annex 4.**

3. MOISTURE ADSORPTION ISOTHERMS AND EVALUATION OF THE EFFECT OF WATER ACTIVITY ON PERMEABILITY, MECHANICAL AND OPTICAL PROPERTIES.

Cellulose-based materials, PVOH and glycerol possess hydrophilic nature, being sensitive to moisture. Hydrophilic materials interact with the environmental moisture, adsorbing or desorbing water molecules from the surrounding environment until to reach the moisture equilibrium with the relative humidity (RH). Water adsorbed interacts by hydrogen bonds with the active polar groups of the polymer chains or components of the matrix. The adsorbed water molecules promote the swelling of the chains and increase the free volume of the matrix, resulting in a plasticizing effect. These physical and structural changes in the polymeric matrix can affect to the functional properties of the films.

Accordingly, the analysis of the moisture content of the samples and the functional properties as function of the environmental conditions are important to develop a complete characterization of the material. This evaluation can help to predict their behavior, suitability or shelf-life based on their application and surrounding environmental conditions.

For this purpose, the moisture adsorption isotherms allow predicting the moisture content of the samples in a wide range of water activity (a_w), and the state of water molecules interaction in polymer matrix.

The moisture adsorption isotherms of regenerated cellulose-glycerol-PVOH, pure BC and BC-glycerol-PVOH films were evaluated following the standardization isotherms method of European Project Group COST 90. The first step was to calculate the moisture content of the samples at different a_w. For that, microclimates were recreated in tightly sealed containers with saturated saline solutions of LiCl, $MgCl_2$, K_2CO_3, NaBr, NaCl and $BaCl_2$ inside a chamber at 30°C to obtain the different RH_{eq} ranged between 11-90.5%. Samples with a side of 3 cm-square were stored in the microclimates for 10 days at 30°C to reach the a_w equilibrium.

The moisture content of the samples with different a_w was calculated gravimetrically by the difference between the weights of the conditioned samples and the dried samples at 105°C. The moisture adsorption isotherm was obtained from the experimental data fitted using the GAB model and the solver plugging of Ms-Excel.

In the literature, previous studies have analyzed the sorption isotherms of several polysaccharides-based films. However, the analyzes of the functional properties of polysaccharide films as function of the moisture conditions have not been limited at a unique standard condition. Modifications of WVP, mechanical and optical properties of

the developed films were studied in wide range of a_w, considering the variation of moisture content of films as a function of RH.

The WVP of pure BC and composite films based on cellulose combined with glycerol and PVOH were evaluated following the standard gravimetric test method E96 at several RH_{eq}. A double bottom wall permeability chamber was designed to recreate different a_w. Several a_w were reached using different RH_{eq} conditions on both sides of the films. Each test was performed by triplicate at 30ºC for 8 h. The weight loss or gained the cup was recorded and plotted as function of time.

Films previously conditioned in different microclimates to have several a_w were used to measure the permeability, mechanical and optical properties depending on the moisture content of the samples.

Mechanical properties of pure BC films and cellulose based films combined with glycerol and PVOH were measured at different a_w using a texturometer (TA-XTplus, Stable Micro System, United Kingdom). Tensile test was carried out using samples of 15×100 mm and following the standard method ASTM D-882. A constant crosshead speed of 0.08 mm/s and initial grip separation of 40 mm were set up. Stress-strain data were recorded during the deformation of the sample to calculate TS, %E and YM.

Puncture test was carried out using samples of 30×30 mm and using a film support platform (HDP/FSR) in the texturometer. A constant

crosshead speed of 1 mm/s until rupture was set up. BS and DB were calculated from the force–deformation curves.

Optical properties of the developed films were measured at several a_w, using a spectrophotometer V-670 (Jasco Inc, Japan). UV-barrier properties, color (L*, a*, and b*), transparency and opacity of the samples were calculated.

ANOVA was used to assess the effect of the water molecules on the properties of the films developed. The mean comparison was performed by Tukey's test ($p < 0.05$), using the software Statistica 7.0.

The methodology of the experiments is described in detail in the section "Material and Methods" of Annex 5 and Annex 7.

3.1. REGENERATED CELLULOSE-GLYCEROL-PVOH BLEND FILMS: EFFECT OF WATER ACTIVITY ON ITS PROPERTIES.

The moisture adsorption isotherms, permeability, mechanical and optical properties of regenerated cellulose-glycerol-PVOH composite films were evaluated. The purpose was to extend the characterization of this formulation film at several moisture contents and analyze the effect of water molecules on its functional properties.

Films with a composition of cellulose 4% (w/w), glycerol 5% (w/w) and PVOH 5%(w/w) showed good values of elasticity, resistance to break, UV-barrier and adsorption properties in previous studies, were selected to evaluate the plasticizing effect of water molecules on their properties.

Figure 1 of Annex 5 shows the moisture adsorption isotherm of the samples. Results indicated that the maximum moisture content of the samples was 20.80% at a_w = 0.90. The presence of glycerol and PVOH resulted in an increase of the maximum content of the samples respect to pure regenerated cellulose. The water molecules are interacting in a different way with the polymer chains. The shape of the isotherm obtained by the GAB model and the GAB parameters could indicate the state of water molecules and the interactions in the films. Experimental adsorption data were well adjusted using the GAB equation (r^2> 0.99). The sigmoidal moisture adsorption isotherm of regenerated cellulose-glycerol-PVOH film could be classified as type II. The values of the GAB parameter were X_m=4.16, C=19.91 and K=0.89. X_m indicates the maximum amount of water molecules that could be adsorbed in a single layer per gram of dry film expressed as percentage. It is a parameter related to the number of adsorption sites. Previous studies reported slightly higher X_m values for regenerated cellulose films from different resources (6.31-7.16 %). The interactions between cellulose-glycerol-PVOH could decrease the number of available active sites on the surface of the film, compared to pure cellulose films. Besides, PVOH decreased the roughness and porosity of the surface of the film, decreasing the surface area and limiting the amount of water on monolayer. Also, the solvent methods to obtain regenerated cellulose films could have effect on the structure of the sample and the interactions with water molecules.

In the adsorption isotherm curve, 3 regions can be identified. First region (a_w = 0.17-0.20) showed a convex shape and corresponds to the water adsorption in the monolayer. In the monolayer, water molecules are linked to the binding sites on the surface of the film, mainly due to the hydroxyl groups of PVOH and glycerol. Second region (0.20 <a_w <0.38) showed a practically linear increase of moisture content to increase a_w. In this region the water adsorption took place in multilayers and it is water partially bound. Third region (a_w> 0.38), it was observed a significant increase of moisture content with a_w. In this region, there were condensed water filling the pores of the polymeric matrix. The condensed water promoted the swelling of the film and the increase of the free volume within the matrix.

The results of the WVP showed values ranged from $5.15 \cdot 10^{-10}$ to $5.44 \cdot 10^{-9}$ g/m·s·Pa, as shown Figure 2 of Annex 5. WVP increased as the RH_{eq} increased. Therefore, the permeability of the regenerated cellulose-glycerol-PVOH films depended on the moisture content of the samples ($p<0.05$). WVP values remained low until a_w = 0.43, where the water adsorption on the multilayer started taking place. At a_w>0.43, the WVP increased exponentially, enduring a significant increase as the RH_{eq} increased. From 0.56 a_w, the multilayers began to saturate and started to appear free water filling the pores in the polymeric matrix. The adsorbed water on the multilayers of the polymer matrix had a plasticizing effect causing a swelling of the polymer matrix and an increase of the free volume. Consequently, the polymer chains suffered

a reorganization, losing the packed structure that eased the water vapour diffusion through the polymeric structure.

Results of the tensile and puncture properties of regenerated cellulose-glycerol-PVOH films are showed in Figure 3 and Figure 4 of Annex 5, respectively. Results showed that the mechanical behavior of the films depended strongly on the moisture content ($p<0.05$). TS values obtained ranged from 92.65 to 17.57 MPa, %E ranged from 5.88 to 19.85% and YM ranged from 3639.09 to 227.89 MPa. The resistance to rupture of the films decreased continuously as the moisture content of the samples increased, observing 3 mainly regions. In the first range (a_w 0.11-0.32), the moisture adsorbed on the monolayer did not have a significant effect on the resistance to rupture of the samples, observing a slightly diminish of TS values. In the second range ($0.43 < a_w < 0.56$), the water molecules adsorbed in the multilayer manifested a plasticizer effect, forcing the reorganization of the polymer chains. In the last section ($a_w>0.56$) there was a significant decrease of TS values, more than 5 folds from the initial value. The water capillary condensation produced a significant plasticizing effect. YM values showed a similar trend than TS, concurring the sections with the most significant modifications. The plasticizing effect of water was also observed in elastic properties. The water adsorbed in the multilayer was responsible for the most significant plasticizing effect, reaching values of 18.61%. However, at a maximum moisture content, the condensed water molecules in the matrix produced an overplastification of the matrix.

As a result, there was a weakening of the structure, decreasing elasticity to 10.76%.

The puncture test results showed BS values ranged from 525.47 to 3420.18 g and DB ranged 0.59-3.97 mm. The BS and DB increased until 0.75 a_w, reaching a value of 3420.18 g and 3.93 mm, respectively. The water adsorbed in multilayer and the free water acted as a lubricant of the polymeric chains, enhanced the resistance of the films subjected to axial deformation. However, from a_w>0.75 the excess of free water in the matrix weakened the structure, decreasing BS values and keeping constant DB values.

Regarding the optical properties, the plasticizing effect of the water molecules affected the optical properties of regenerated cellulose-glycerol-PVOH samples. Results of transmittance values in the UV region, color, transparency and opacity are shown in Table 1, Table 2 and Figure 5 of the Annex 5. In the UV region, transmittance values were ranged between 5.36-13.25, 4.25-10.02 and 2.09-4.82 % for UV-A, UV-B and UV-C regions, respectively. The transmittance values increased slightly to increase a_w, up to 0.57. The behavior changed at higher a_w, decreasing to reach similar initial values. Probably, the initial structural modifications due to the plasticizing effect of water resulted in a slight increase of the transmittance values. At higher a_w, the condensed water into the voids of the films diminished the transmittance values. Probably, due to the increase of the thickness promoted by the swelling of the chains and the optical properties of the

water itself. Transparency values ranged between 11.30 and 18.67 meanwhile opacity values ranged from 10.18 to 14.37. The color parameters L*, a* and b*, showed similar tendency than the transmittance in the UV region. Lightness, redness and yellowish parameters increased up to 0.57 a_w, followed by a decrease until reaching similar values than the initial values.

Justification, results and discussion of this section are extensively detailed in **Annex 5.**

3.2. PURE BACTERIAL CELLULOSE FILMS: EFFECT OF WATER ACTIVITY ON ITS PROPERTIES.

The exceptional 3D structure and high purity presented by BC, unlike regenerated cellulose, makes cellulose a material of great interest for a wide application in several fields. Understanding the moisture adsorption mechanism in BC films and the water effect on its functional properties are essential to develop and reformulate new materials with improved properties. Hence, the water effect analysis could led to extend the applications of bacterial cellulose in biomedical, cosmetic, food industry or agriculture field.

The calculated moisture adsorption isotherm of BC at 30ºC is showed in Figure 1 of Annex 6. The moisture content of BC depended on the a_w, increasing to increase the a_w. BC samples reached the highest moisture content of 25.86% at a_w = 0.90, higher than the values showed by pure regenerated cellulose. Probably, the microstructural differences

between bacterial cellulose and regenerated cellulose were the reason of the moisture content difference. Previous research indicated that the adsorption properties of cellulose depended on the void system (diameter, volume and inner surface of voids). Accordingly, the porosity of BC benefited the physical retention of water molecules inside the polymer matrix. Experimental adsorption data was well fitted using the GAB equation (r^2>0.98), being X_m=3.82, C=12.00 and K=0.95. X_m value of BC was lower than the values reported for regenerated cellulose. The difference of X_m values was due mainly to two factors. First, the difference in crystallinity of celluloses depending on the origin, the crystallinity of BC being greater than the crystallinity of the regenerated cellulose. Crystalline regions in the cellulose matrix are inaccessible to water molecules, being the amorphous region where water molecules are adsorbed. Besides, the methods to obtain regenerated cellulose films and the different sources could affect the structure of the film, modifying its properties.

The moisture adsorption isotherm could be classified as type II with three main regions. The first region (a_w<0.25), the isotherm showed a convex shape. In this region the water was strongly linked to the active groups, forming the monolayer adsorbed water. The second region was up to 0.55 a_w and the isotherm showed a linear increase of the moisture content to increase the a_w. In this region, the multilayer was formed where the particles are partially linked. From a_w>0.55, a

significant increase was observed with the a_w, due to the condensed water that filled the voids of the polymer structure.

The structure alterations affected to the diffusion properties of water molecules throughout the film, showing different WVP properties as function of a_w. Figure 2 of Annex 6 shows the WVP values obtained for BC films at several a_w. Results showed WVP values of BC ranged from $1.35 \cdot 10^{-12}$ to $3.13 \cdot 10^{-11}$ g/m·s·Pa. WVP values showed a significant increase by increasing the a_w, up to 0.48 a_w. The variation of the permeability phenomenon of BC meant that structure modifications were induced during the adsorption of water molecules in the multilayer. The water adsorbed in the multilayer acted as plasticizer, swelling the polymer chains and opening the structure due the increase of the free volume. This behavior changed from a_w>0.48, keeping the values with small variations. Probably, due to the high crystallinity of BC, above 0.48 a_w, the active sites where water molecules could interact were limited by the water adsorbed in the monolayer and multilayer, softening the variations in permeability values at higher a_w.

Regarding mechanical properties, Figure 3 and Figure 4 of Annex 6 show the data obtained in tensile and puncture test, respectively. Results indicated that the mechanical properties of BC films were strongly affected by a_w ($p<0.05$). TS values were ranged from 15.50 to 22.28 MPa. The lowest TS value was reached at 0.11 aw. In this case, the plasticizer effect of water was observed at higher values of a_w, decreasing the TS meanwhile increasing a_w. The elasticity properties

showed %E values ranged between 1.36-3.71%. The elongation of the BC films increased throughout the evaluated a_w range to increase a_w. The MY values calculated were ranged between 609.12-1426.77 MPa. MY showed similar trend to TS based on a_w. Water manifested a plasticizer effect at a_w>0.43, showing a continuous decrease of the values to increase a_w.

The puncture test indicated BS values ranged between 145.03 - 338.10 g and DB values ranged from 0.39 to 1.86 mm, as shown Figure 4 of Annex 6. Both puncture properties showed the same tendency, increasing the values by increasing a_w. In this case, the water molecules enhanced the mobility among the polymer chains. Hence, water eased the reorganization of the polymer chains and resistance during the axial deformation, resulting in an increase of the puncture values to increase the moisture content of the samples.

The good UV-barrier properties of BC became this material a potential material to apply in direct contact with food products. The UV-barrier and optical properties of BC can suffer modification due to the moisture content and the water-polymer interactions. Figure 5 and Table 1 of Annex 6 show the results of transmittance values of BC films at several a_w.

The optical properties of BC were analyzed at lower (0.11 a_w), intermediate (0.33 and 0.57 a_w) and higher (0.91 a_w) values of a_w. The BC transmittance data ranged 2.48-5.61 %, 0.33-2.10 % and $3.75 \cdot 10^{-3}$-$4.95 \cdot 10^{-1}$ % in the UV-A, UV-B and UV-C range, respectively. Results

indicated excellent protective properties against the UV radiations. The lower transmittance value was reached at higher moisture content, due to the water's own adsorbent properties in the UV region.

Table 2 of Annex 6 shows the transparency and opacity parameter calculates and Table 3 of Annex 6 shows the color parameters obtained. Transparency values ranged from 21.34 to 27.42 and opacity values from 18.33 to 28.65. There were not higher variations between the values of samples with different moisture content. However, bacterial cellulose films had a smoked color, presenting only a good visual appearance when it was in direct contact with a surface. Regardless the parameter lightness (L* value), redness (value a*) and yellowish (b* value) data did not show important variations that could affect the visual perception of the consumers, as shown the data of the Table 3 of Annex 6.

Justification, results and discussion of this section are extensively detailed in **Annex 6.**

3.3. BACTERIAL CELLULOSE - GLYCEROL - PVOH BLEND FILMS: EFFECT OF WATER ACTIVITY ON ITS PROPERTIES.

The moisture adsorption isotherms and the effect of a_w on permeability, mechanical and optical properties of films based on BC combined with PVOH and glycerol were evaluated. It was selected the film formulation that showed better mechanical and optical properties based on the results of previous works, as shown Figure 1 of Annex 7.

The select films were BC combined with glycerol 5%(w/w) and PVOH 5%(w/w).

As shown the moisture adsorption isotherm of Figure 2 of Annex 7, the highest moisture content of BC-glycerol-PVOH was 62.85% at a_w=0.9. The presence of glycerol and PVOH produced an important increase of this value with respect to pure cellulose. The increase of hydroxyl groups due to the presence of glycerol and PVOH produced the increase of the amount of water in the samples at higher a_w. The BC-glycerol-PVOH isotherm showed a slow initial increase of the moisture content to increase to 0.57 a_w. Above a_w>0.57, a strong increase of moisture content was observed by increasing a_w.

BC-glycerol-PVOH isotherm was type III, according to the Brunauer classification. Isotherms types III describe adsorption on macroporous adsorbents with weak adsorbate-adsorbent interactions. As result, the interaction of adsorbate with an adsorbed layer is greater than the interaction with the adsorbent surface. Adsorbent-adsorbate interactions are now relatively weak and adsorbed molecules are grouped around the most favorable sites on the surface of the film. Therefore, there was no identifiable monolayer formation. Type III sorption isotherm is characteristic of components rich in hydrophilic components such glycerol.

Experimental adsorption data were well fitted using the GAB equation (r^2 > 0.99). The GAB parameter values were X_m = 13.80, C =

1.17 and K = 0.91. X_m parameter indicated that the presence of glycerol and PVOH increased the capacity to retain moisture in the monolayer.

The WVP of hydrophilic materials depends on the a_w, unlike synthetic polymers which the permeability coefficient is independent from a_w.

The WVP permeability of BC-glycerol-PVOH films were measured in a wide range of a_w at 30 ºC and the obtained values were ranged between $6.50 \cdot 10^{-9}$-$2.71 \cdot 10^{-8}$g/m·s·Pa. Figure 3 of Annex 7 shows the WVP values and trend as function of a_w, observing two regions or permeability trends. Fist, at lower water vapour pressure, the permeability values of the samples increased by increasing the a_w, up to 0.45 a_w, reaching the highest WVP value. In this region, the plasticization of amorphous regions, the swelling of the polymer chains and the increase of the free volume occurred. Internal changes and water-polymer interactions promoted the reorganization of the structure, which resulted in a more open structure that eased increased flow.

Above 0.45 a_w, WVP suffered a slightly decrease of the permeability values, up to a value of $1.96 \cdot 10^{-8}$ g/m·s·Pa. This permeability behavior was unusual, because generally the permeability of polysaccharide films increases to increase the a_w. Probably, the hygroscopic properties of glycerol and the strong interaction glycerol-water molecules at a_w hindered the permeance, resulting in a slight decrease of WVP values. The strong hygroscopic character of glycerol

was observed in films at high a_w, producing a condensation of water on the surface of the film, as shown Figure 4 of Annex 7. These condensation phenomena could be responsible for the variations between the permeability measures to higher a_w.

Mechanical properties analysis at several a_w indicated that films were strongly affected by a_w ($p < 0.05$), as shown Figure 5 and Figure 6 of Annex 7. Tensile test showed results values for TS ranged between 1.16-6.57 MPa, %E ranged between 8.51-34.08% and YM ranged between 10.31-70.54 MPa. The resistance to rupture suffered a continuous decrease to increase a_w of the samples, being more significant at a_w values above 0.57. YM values showed similar trend than TS, with a continuous decrease to increase a_w. The trend observed in TS and YM responded to the typical behavior of these properties with the incorporation of a plasticizer to the matrix. Regarding elasticity properties, %E values increased slightly to increase a_w up to 0.32, as result of the water plasticizing effect and in concordance with the results obtained in the isotherms and permeability properties. Nevertheless, at higher a_w values (a_w>0.56), %E was drastically reduced until 8.51%. At high a_w values, the samples were overplasticized, resulting in a weak structure and decreasing drastically the elongation.

Puncture test showed BS values ranged between 299.92 and 1943.26 g and DB values ranged from 2.56 to 4.03 mm. BS showed the same trend as resistance to rupture, a continuous decrease was observed, being more acute from a_w values above 0.57. However, the

puncture elasticity did not show a significant or drastic decrease. In this case, the water molecules that acted as a lubricant between the polymer chains did not affect the resistance of the films subjected to axial deformation.

The UV-barrier properties of the developed films at several a_w are shown in Figure 7 and Table 1 of Annex 7. The developed films possess excellent protective properties against UV radiation, mainly due to the properties of cellulose and glycerol. The values obtained were ranged between 3.25-0.50%, 13.02-2.94% and 27.66-10.81% in the UV-C, UV-B and UV-A regions. The transmittance values diminished when the moisture content or a_w of the films increased. Probably, the structural changes resulted the plasticizing effect of water, the swelling of the chains and the water's own UV-light adsorption properties were the factor that produced the decrease of the transmittance values to increase a_w.

Regarding the color properties, the results obtained for L*, a* and b* parameters and the transparency and opacity values are shown in Table 2 of Annex 7. The samples showed a translucent and shiny appearance at the studied moisture contents. Although, they became slightly opaque at high moisture contents probably because the hygroscopic properties of the glycerol promoted the adsorption of water molecules. Regardless of the color parameters, the lightness (L* value), redness (value a*) and yellowish (b* value) parameter did not show significant variations until 0.75 aw. However, a significant change was

observed at the highest aw value; probably the high content of condensed water on the surface of the film interfered with the transmittance measurements of the film.

Justification, results and discussion of this section are extensively detailed in **Annex 7.**

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CHAPTER IV – CONCLUSIONS

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CHAPTER IV – CONCLUSIONS

1. It is feasible to obtain cellulose-based films, from vegetable or bacterial sources, with improved functional properties by combining with glycerol and PVOH. By the combination with glycerol and PVOH, it is possible to overcome the cellulose limitations and extend its potential applications in food packaging.

2. The porosity and structure of cellulose films allowed the diffusion of PVOH through the polymer matrix by immersion in PVOH baths, resulting in a reinforcing agent.

3. The reinforcing effect of PVOH enhanced the mechanical properties of cellulose samples, showing superior elasticity and resistance properties.

4. Glycerol manifested a plasticizing effect on BC films, improving the elasticity properties of the samples, and "anti-plasticizing" effect on regenerated cellulose films.

5. The WVP values obtained from the developed films were 2 orders of magnitude higher from those values from synthetic polymers.

However, cellulose composited films showed lower WVP values than permeability values from pure PVOH films.

6. Cellulose composite films showed low transmittance values in the UV region, indicating good optical barrier properties against UV-radiation. The UV-barrier properties were improved by the addition of glycerol. PVOH enhanced the visual appearance of the samples, increasing the transparency and decreasing the opacity values.

7. FT-IR, DSC and TGA analysis indicated physical interactions between cellulose-glycerol-PVOH, which gave composite films with higher thermal stability than pure polymers.

8. The mechanical, water permeability and optical properties of the films depended on their moisture content and the relative humidity of the surrounding environmental.

9. Water manifested a plasticizing effect on cellulose-glycerol-PVOH films, suffering an "overplastification" at high relative humidity conditions.

10. The complete characterization of the developed composite in a wide range of a_w, will allow knowing the suitability of the material for a certain application depending on the storage conditions.

11. The study of adsorption isotherms of pure BC, and the plasticizing effect of water on its functional properties may provide new knowledge about the behaviour of this material that allows future reformulations to improve its properties and to obtain materials with potential applications such as active packaging.

12. Cellulose-glycerol-PVOH composite films could be useful as an alternative to synthetic films to protect foods against UV-light radiations in order to retard the occurrence of oxidation reactions.

13. Cellulose-based films showed high water retention capacity values, being an alternative to develop adsorbent pads for direct contact with foods based on cellulose.

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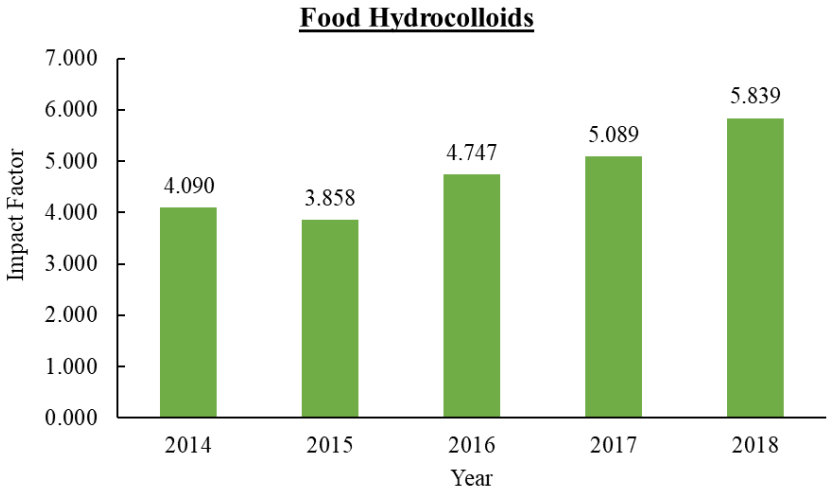
PUBLICATION 1. NOVEL COMPOSITE FILMS FROM REGENERATED CELLULOSE – GLYCEROL - POLYVINYL ALCOHOL: MECHANICAL AND BARRIER PROPERTIES (ANNEX 1)

Journal: Food Hydrocolloids
Publisher: Elsevier Ltd.
ISSN: 0268-005X
Country: Netherlands

Reference: Cazon, P., Velazquez, G., & Vázquez, M. (2019). *Novel composite films from regenerated cellulose-glycerol-polyvinyl alcohol: Mechanical and barrier properties*. Food Hydrocolloids, 89, 481–491. https://doi.org/10.1016/J.FOODHYD.2018.11.012

Journal Metrics:
Category and rank (2018): Food science & technology: 5/135
Impact factor (2018): 5.839
Quartile: Q1



PUBLICATION 2. CELLULOSE – GLYCEROL - POLYVINYL ALCOHOL COMPOSITE FILMS FOR FOOD PACKAGING: EVALUATION OF WATER ADSORPTION, MECHANICAL PROPERTIES, LIGHT-BARRIER PROPERTIES AND TRANSPARENCY (ANNEX 2)

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Journal Metrics:
Category and rank (2018): Polymer science: 4/87
Impact factor (2018): 6.044
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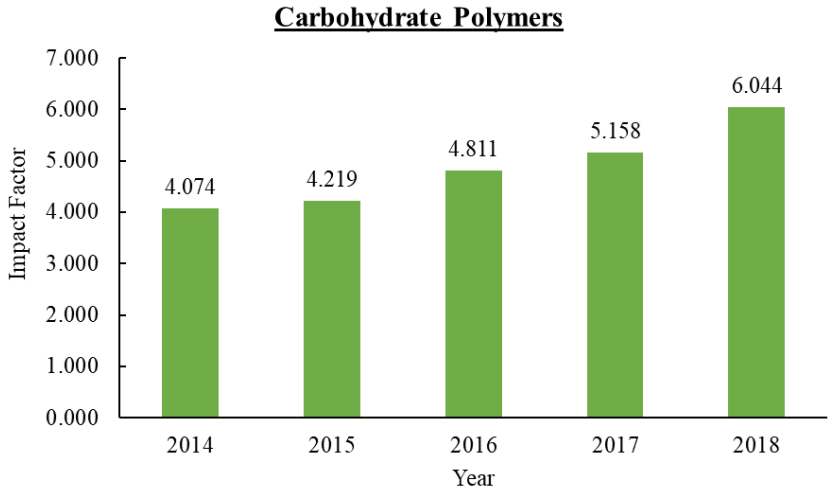

PUBLICATION 3. CHARACTERIZATION OF MECHANICAL AND BARRIER PROPERTIES OF BACTERIAL CELLULOSE, GLYCEROL AND POLYVINYL ALCOHOL (PVOH) COMPOSITE FILMS WITH ECO-FRIENDLY UV-PROTECTIVE PROPERTIES (ANNEX 3)

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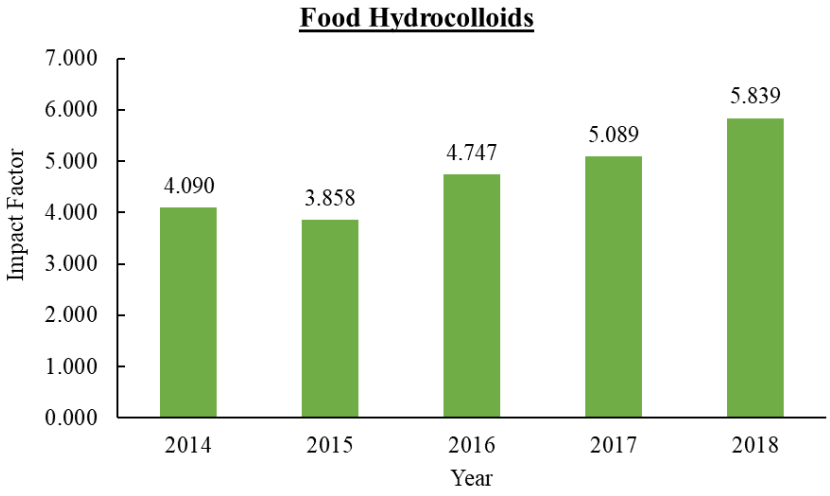

PUBLICATION 4. COMPOSITE FILMS WITH UV-BARRIER PROPERTIES OF BACTERIAL CELLULOSE WITH GLYCEROL AND POLY(VINYL ALCOHOL): PUNCTURE PROPERTIES, SOLUBILITY, AND SWELLING DEGREE (ANNEX 4)

Journal: Biomacromolecules
Publisher: American Chemical Society
ISSN: 1526-4602
Country: USA

Reference: Cazon, P., Vázquez, M., & Velazquez, G. (2019). *Composite Films with UV-Barrier Properties of Bacterial Cellulose with Glycerol and Poly(vinyl alcohol): Puncture Properties, Solubility, and Swelling Degree*. Biomacromolecules, 20(8), 3115–3125. https://doi.org/10.1021/acs.biomac.9b00704

Journal Metrics:
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Impact factor (2018): 5.667
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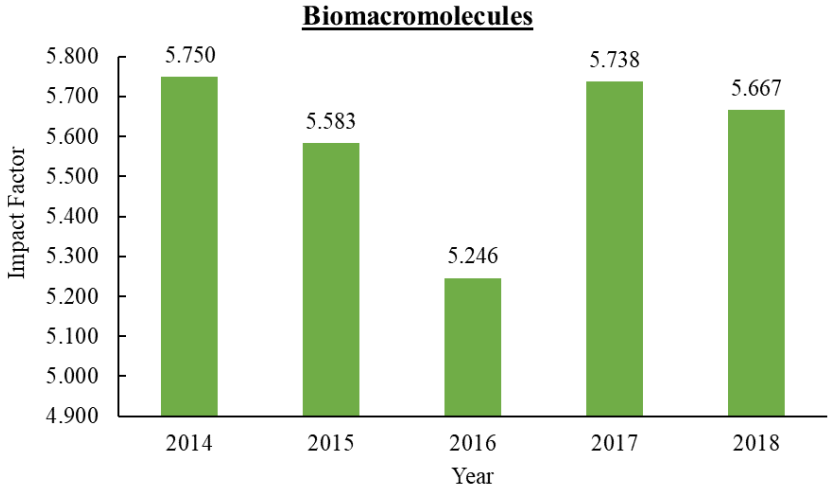
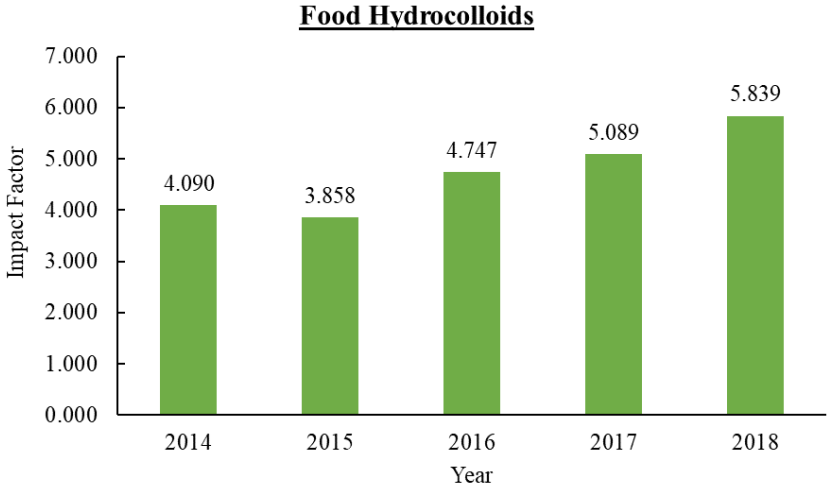


PUBLICATION 5. REGENERATED CELLULOSE FILMS COMBINED WITH GLYCEROL AND POLYVINYL ALCOHOL: EFFECT OF MOISTURE CONTENT ON THE PHYSICAL PROPERTIES (ANNEX 5)

Journal: Food Hydrocolloids
Publisher: Elsevier Ltd.
ISSN: 0268-005X
Country: Netherlands

Reference: Cazón, P., Velázquez, G., & Vázquez, M. (2020). *Regenerated cellulose films combined with glycerol and polyvinyl alcohol: Effect of moisture content on the physical properties*. Food Hydrocolloids, 103, 105657.
https://doi.org/10.1016/J.FOODHYD.2020.105657

Journal Metrics:
Category and rank (2018): Food science & technology: 5/135
Impact factor (2018): 5.839
Quartile: Q1

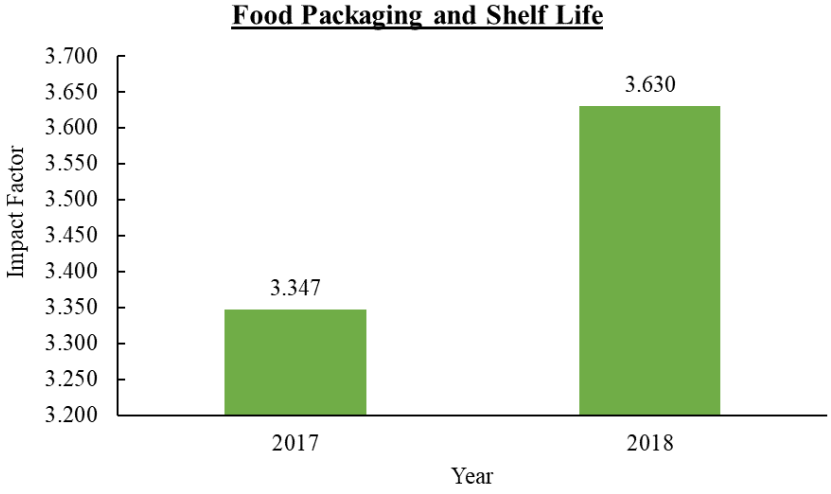


MANUSCRIPT 6. BACTERIAL CELLULOSE FILMS: EVALUATION OF THE WATER INTERACTION (ANNEX 6)

Authors: Patricia Cazón, Gonzalo Velázquez, Manuel Vázquez
Journal: Food Packaging and Shelf Life
Publisher: Elsevier Ltd.
ISSN: 2214-2894
Country: Netherlands

Reference: Cazón, P., Velázquez, G., & Vázquez, M. (2020). *Bacterial cellulose films: evaluation of the water interaction.* Food Packaging and Shelf Life, submitted 8-2-20.

Journal Metrics:
Category and rank (2018): Food science & technology: 25/135
Impact factor (2018): 3.630
Quartile: Q1



MANUSCRIPT 7. UV-PROTECTING FILMS BASED ON BACTERIAL CELLULOSE, GLYCEROL AND POLYVINYL ALCOHOL: EFFECT OF WATER ACTIVITY ON BARRIER, MECHANICAL AND OPTICAL PROPERTIES (ANNEX 7)

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Reference: Cazón, P., Velázquez, G., & Vázquez, M. (2020). *Uv-protecting films based on bacterial cellulose, glycerol and polyvinyl alcohol: effect of water activity on barrier, mechanical and optical properties.* Carbohydrate Polymers, submitted 27-1-20.

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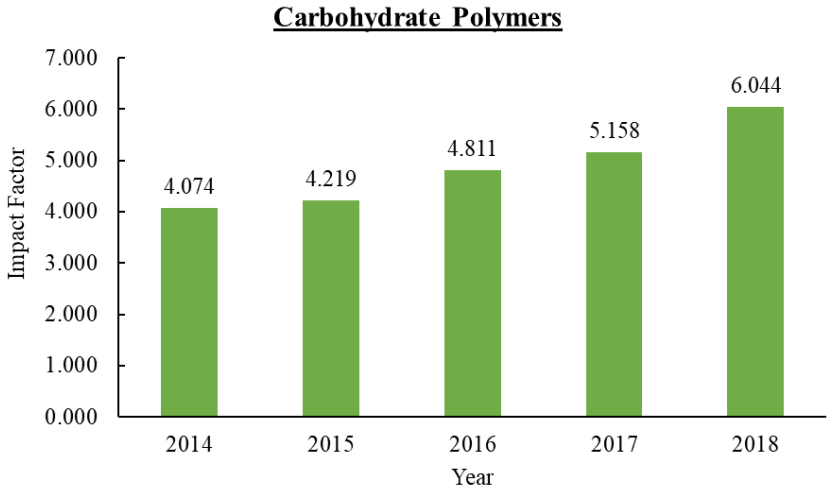

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